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MYCOLOGIA

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VOL. XXXVIII JANUARY–FEBRUARY, 1946 NO. 1

A CLAMP-BEARING FUNGUS PARASITIC AND PREDACEOUS ON NEMATODES

CHARLES DRECHSLER [1]

(WITH 7 FIGURES)

Among 10 Hyphomycetes which in a paper (12) published 4 years ago I set forth as attacking nematodes after the manner most familiar in such fungous parasites, that is, through invasion by means of hyphae resulting from the germination of conidia affixed to the animal host, were included 2 species whose filaments bore clamp-connections characteristic of various groups within the Basidiomycetes. Owing to some minor differences, particularly in their sterigmata, the 2 species could not both be aptly referred to any one mucedinaceous genus then known. As assignment to separate genera would almost certainly have obscured the intimate kinship of the 2 species, a new genus, *Nematoctonus,* was erected for them; this disposition being deemed all the more advantageous since it would serve to bring into relief how unusual the biological relationship here concerned—parasitism on animals normally free-living and motile from the moment of hatching until the approach of death—was among fungi belonging in the Basidiomycetes. The diagnosis of the new genus was intentionally phrased so as to make provision not only for the 2 parasitic species then described under

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the binomials *N. tylosporus* and *N. leiosporus,* but also for a third form generally similar to them in its production of conidia and clamp-connections, as well as in its habitual subsistence on nematodes, but differing significantly in attacking eelworms for the most part in a predaceous manner—that is, by holding the animals through adhesion to specialized vegetative organs, eventually to extend into the helpless captives assimilative hyphae of mycelial origin. Since the third form had been available for examination only in cultures obtained from Hawaii, which when received by ordinary mail were too old to allow preparation of satisfactory figures, it was left unnamed in the hope that an accession of younger material might later make possible a better description. This hope has so far not been fulfilled, owing to failure of the fungus to appear in such cultures as I have subsequently had occasion to prepare from decaying plant detritus collected mostly in Virginia, Maryland, Delaware, Maine, Wisconsin, and Colorado; though 2 additional clamp-bearing species parasitic on nematodes have come to light and have been described (13) under the binomials *N. pachysporus* and *N. leptosporus.* More recently, again, a sixth member of the genus, which, besides attacking eelworms parasitically, operates very destructively in a predaceous manner much like the Hawaiian species, has been observed under favorable conditions and in adequate abundance.

The fungus in question was obtained from 2 collections of friable vegetable refuse gathered by W. J. Zaumeyer near Greeley, Colorado, in October, 1944; one of the collections consisting mainly of partly decayed cucumber (*Cucumis sativus* L.) vines and partly decayed lilac (*Syringa* sp.) leaves, while the other was composed largely of decayed remnants of tamarisk (*Tamarix* sp.) leaves, cottonwood (*Populus* sp.) leaves, and oleaster (*Elaeagnus angustifolia* L.) leaves. In accordance with routine procedure pinches of material from both collections were planted in Petri dishes on maizemeal agar already well permeated with oömycetous mycelium; opportune utilization being made, in this instance, of old cultures of *Pythium arrhenomanes* Drechsl. and *P. undulatum* Petersen *sensu* Dissmann. Before long a flourishing population of eelworms was present in all the cultures, with the result that after about 10 days the predaceous hyphomycetes *Arthrobotrys*

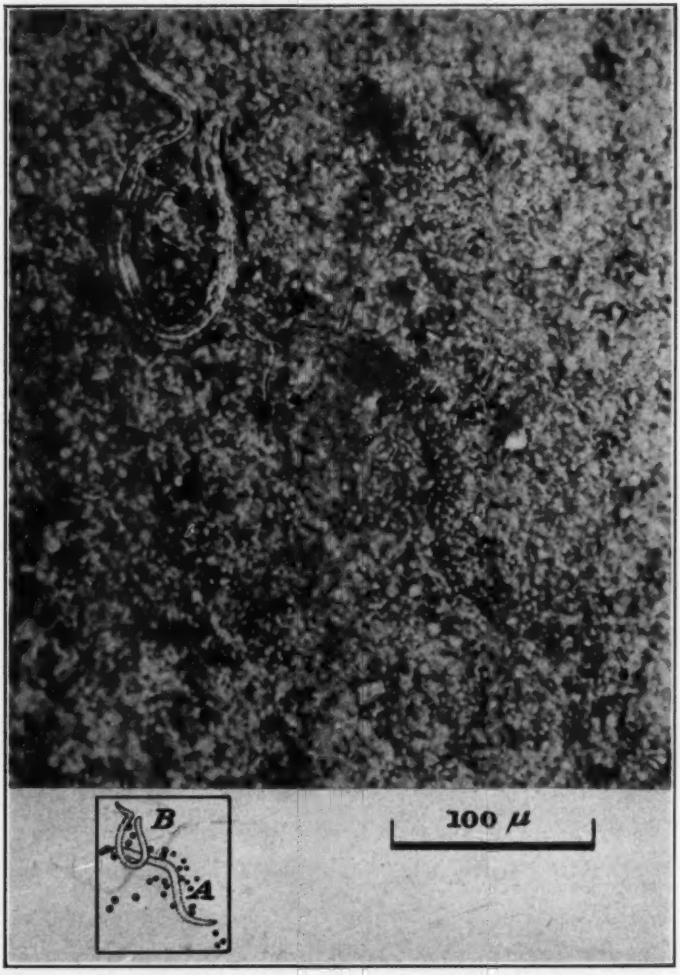

FIG. 1. *Nematoctonus haptocladus* × 300.

oligospora Fres. and *A. arthrobotryoides* (Berl.) Lindau, as also the hyphomycetous parasite *Harposporium anguillulae* Lohde, were developing extensively. Usually it was not until these familiar hyphomycetes had been active for several days that the new clamp-bearing fungus could be found beginning to destroy eelworms in numbers sufficient to arrest attention, though its destructiveness in many cultures soon became very severe. A nematode identified as an undescribed species of *Panagrolaimus*,[2] which predominated numerically over all other species, was often found killed in spectacular quantity. A stylet-bearing nematode identified as *Paraphelenchus pseudoparietinus* Micoletzky [2] and several species of the genera *Rhabditis* and *Mononchus* incurred destruction in a measure corresponding approximately to their lesser abundance.

While as a general rule the predaceous fungi, including both the nematode-capturing hyphomycetes and the nematode-capturing Zoöpagaceae, begin their visible development in Petri plate cultures containing deposits of decaying plant material by extending mycelial filaments from the opaque deposits into the surrounding transparent agar medium, the clamp-bearing fungus more often makes its initial appearance in growing out of dead nematodes (FIG. 1, *A*) lying at some distance—frequently 10 to 20 mm.—from the nearest mass of vegetable detritus. In their earlier stages of development the mycelial filaments growing out into the clear culture medium offer no unusual features (FIG. 2, *A*). Except for the cross-walls associated with the clamp-connections studding them at moderate intervals, they contain few septa. The longer hyphae show moderate branching at rather wide angles; some, though not all, of the branches manifestly arising from individual clamps. Neither vacuoles nor granular constituents are at all abundant in young hyphae; so that the protoplasmic contents present a rather clear, nearly homogeneous appearance.

The commonplace morphology just described pertains more especially to mycelial elements formed under the surface of agar substratum, and consequently is displayed on a greater scale in

[2] For identification of these species I am indebted to Dr. G. Steiner, Principal Nematologist, Division of Nematology, Plant Industry Station, Beltsville, Maryland.

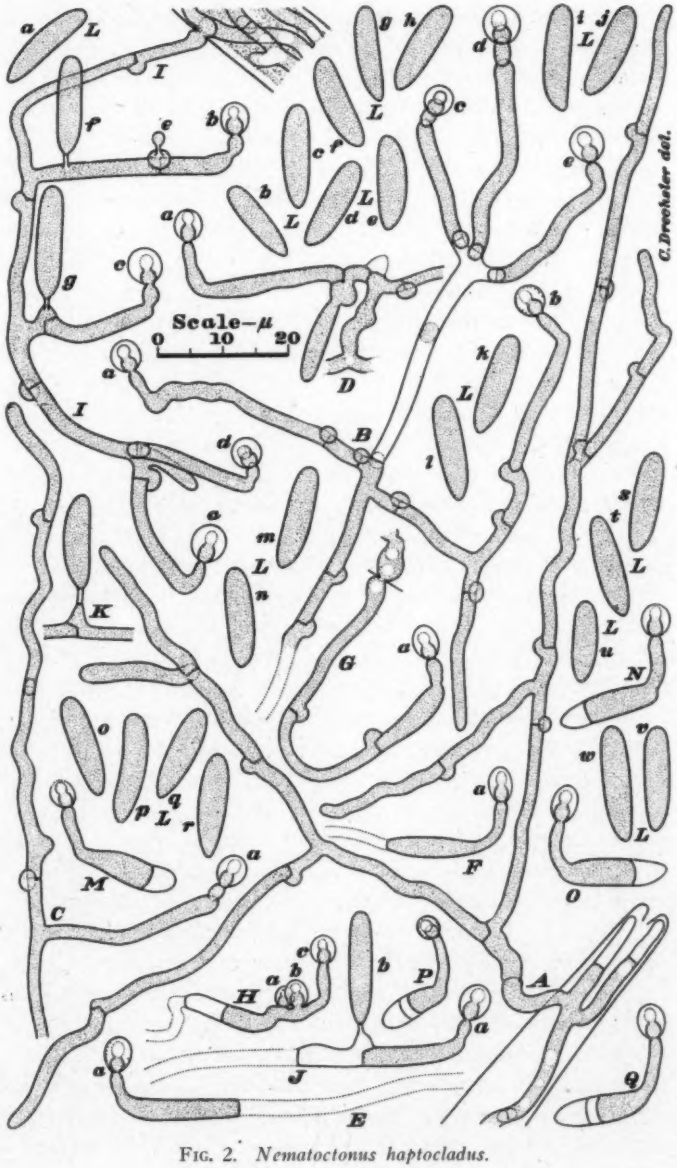

FIG. 2. *Nematoctonus haptocladus.*

instances where the nematode has happened to succumb in a deeply submerged position than in instances where it succumbed on the surface. Once the hyphae from a submerged animal reach the surface they continue their development for the most part procumbently, and then are capable of undergoing the same distinctive modification (FIG. 2, *B*) which hyphae growing out from animals lying on the surface can achieve with little preliminary expenditure of their substance (FIG. 3, *A, B*). To accomplish this modification the prostrate axial filament, as also its prostrate branches, stops elongating procumbently, and gives rise abruptly from its tip to a short, erect or ascending process terminating in a glandular cell that soon is surrounded by a transparent adhesive secretion (FIG. 2, *B, a–e; C, a; D, a; E, a; F, a; G, a; H, a–c; I, a–d.* FIG. 3, *A, a–g; B, a, b; C, a; D, a–j.* FIG. 4, *A, a, b*). The glandular cell usually has a shape somewhat like the Arabic numeral 8. Generally its proximal lobe is noticeably wider than its distal lobe, and appears to be surrounded by thicker, more substantial membrane. As the boundary of the distal lobe, especially at the rounded tip, is often only vaguely discernible, it may be surmised that this lobe is the more directly active one in the elaboration of adhesive material. When viewed under the microscope, in a moist preparation covered with a cover glass, the adhesive drop shows a distinct boundary, as if it were surrounded by a thin peripheral film. Sometimes short rod-like markings appear to extend radially inward from the peripheral contour of the droplet (FIG. 2, *E, a.* FIG. 3, *A, a–g; B, a; D, e–j*). It is quite possible that these markings may represent only minute folds in the peripheral film such as might readily result from the pressure of the overlying cover glass. Not infrequently a predaceous branch, after putting forth one adhesive body, will elongate slightly to put forth a second (FIG. 3, *A, b, c; D, h, i.* FIG. 4, *A, a, b*); and occasionally elongation is repeated in the development of a third adhesive body (FIG. 2, *H, a, b, c*). This rejuvenation of predaceous branches has an obvious parallel in the repeated elongation of the outgrowth put forth by abjointed conidia of *Nematoctonus pachysporus*.

As the glandular cells with their envelopes of adhesive secretion are borne aloft in the air 2 to 8 μ above the substratum they stand out in bold relief when cultures containing them are examined

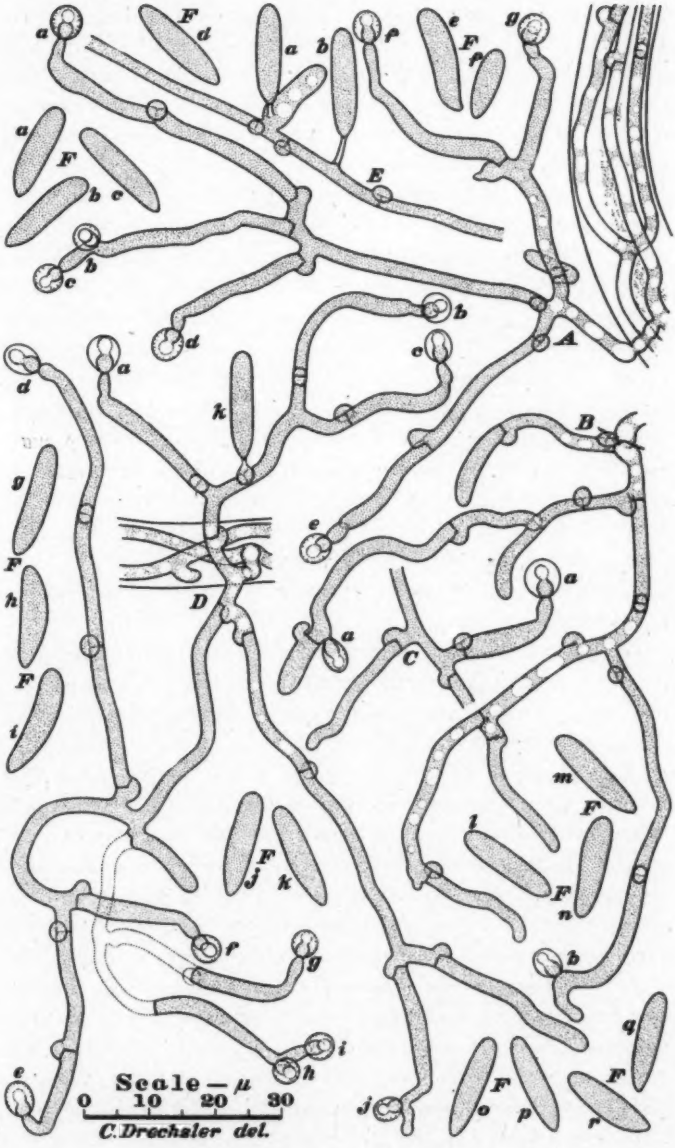

FIG. 3. *Nematoctonus haptocladus.*

microscopically in an uncovered state by means of a dry objective (FIGS. 1, 5, 6). Owing to their raised position they adhere only to nematodes moving along on the surface of the culture. Small eelworms, as, for example, young specimens of the undescribed *Panagrolaimus* species mentioned, are usually held securely despite all struggles, even when attached only by a single adhesive branch (FIG. 7, *A, a*); and somewhat larger animals are frequently held captive if two (FIG. 1, *B*) or more branches become fastened upon them. Like the nematode-capturing Zoopagaceae, but unlike most of the clampless hyphomycetous forms familiarly exemplified in *Arthrobotrys oligospora,* the fungus shows no special development operative in killing or disabling quickly any nematode captured by it. It accordingly delays invasion—certainly invasion on an extensive scale—until the animal has become quiescent from prolonged struggle. A small specimen of *Paraphelenchus pseudoparietinus,* whose capture happened to come under direct observation, remained capable of some slight movement fully 31 hours later. Forty-eight hours after capture the animal was motionless (FIG. 7, *B*), though death had probably taken place only a short time earlier, judging from the meager display of assimilative hyphae inside its body.

While the smaller, weaker nematodes are usually held captive on the adhesive branches, the larger and stronger animals more often tear these branches away, and continue moving about, carrying the adhesive organs with them (FIG. 7, *C, a–f*). Such escape, however, brings little advantage, for the affixed fungous structures penetrate the integument and intrude infective hyphae, eventually killing the fugitives, much as if they had been held captive. Because of the long period required to bring about their disablement, the fugitive animals roam about widely, often entering other areas of infestation and thus becoming burdened with additional predaceous apparatus. In cultures where the fungus was abundant, large specimens of the unnamed *Panagrolaimus* measuring .75 to 1 mm. in length could often be seen encumbered with 15 to 20 adhesive branches; and much more frequently somewhat less robust specimens were found still moving about feebly, though a half-dozen adhesive branches had intruded assimilative hyphae of some length (FIG. 7, *C, a–f*). Infected eelworms, thus capable of

prolonged locomotion are, of course, wont to succumb in scattered positions, much like eelworms killed by fungi attacking wholly after the ordinary manner of parasites. Its failure to hold the more robust nematodes not only explains largely why the fungus often makes its first appearance in Petri plate cultures at a distance from the deposit of decaying material whence it must have originated, but also explains for the most part why nematodes are often found succumbing to it in submerged positions, despite its production of predaceous organs only on the surface of the agar substratum. Again, partly because of the limited mechanical strength of the filaments terminating in adhesive organs, and partly because of the limited elongation of hyphae extended from dead nematodes, flourishing cultures of the fungus show a characteristic distribution of dead and dying eelworms in numerous colony-like groups —the groups including often 5 to 25 small eelworms held captive through adhesion to the abundant predaceous apparatus originating from the body of a conspicuously larger eelworm that had earlier succumbed to invasion from predaceous apparatus which it manifestly had torn off but had not succeeded in shedding.

There are grounds for believing that the frequent tearing away of adhesive organs by the stronger eelworms is a normal and more or less advantageous feature in the development of the fungus. Often a ramifying predaceous hyphal system, still in a fairly youthful condition, undergoes evacuation of protoplasm from intercalary portions, with the result that the adhesive branches distal in relation to these portions remain attached only very weakly by empty membranes so unsubstantial as to be hardly visible (FIG. 2, *B, c, d, e; E; H; J.* FIG. 3, *D, g, h.* FIG. 7, *A, c, d; B, a, b*). Through weakening of the hyphal attachments the fungus achieves a condition analogous to that found in 3 species of the clampless nematode-capturing hyphomycetes—*Dactylella lysipaga* Drechsl., *Dactylella leptospora* Drechsl., and *Dactylaria candida* (Nees) Sacc.—whose predaceous organs, consisting of adhesive knobs and non-constricting rings, are borne on longish, slender, and evidently frail filamentous stalks. As was set forth in an earlier account (**8**: p. 499–508; p. 523–527), the rings of these species are often torn from their attachments, permitting the ensnared eelworm to proceed on its way. Since the earlier account was written I have

had occasion further to observe in cultures of *Dactylella lysipaga* and *Dactylaria candida* nematodes moving about with predaceous knobs of these fungi adhering externally to them; the knobs in time infecting the encumbered animals and bringing about their death, after a considerable period of motility, in widely scattered positions. Such an extended period of motility after predaceous organs—whether adhesive knobs or non-constricting rings—have been affixed to prey would seem very helpful in spreading the fungus; so that it may well be significant that the 3 clampless hyphomycetes named, much like the clamp-bearing form from Colorado, but wholly unlike most other clampless nematode-capturing hyphomycetes, are not wont to kill eelworms quickly. The frail attachment of predaceous branches consequent to withdrawal of protoplasmic contents from intercalary hyphal parts may thus with some justification be regarded as an adaptation not only advantageous in allowing development of additional predaceous branches from the protoplasmic materials withdrawn, but profitable, moreover, in utilizing the animal's locomotion to extend the region of infestation. It must be admitted, however, that similar extension, though on a lesser scale, would seem to be accomplished quite fortuitously now and then in behalf of clampless hyphomycetes whose predaceous organs are not only attached very firmly to the mycelium but, besides, operate in a manner calculated to inflict death quickly; as, for example, in instances when powerful specimens of *Rhabditis* or *Mononchus*, after tearing off, through sheer violence, the firmly attached constricting rings of *Arthrobotrys dactyloides* Drechsl., *Dactylella bembicodes* Drechsl., or *Dactylaria brochopaga* Drechsl., continue their locomotion for an hour or two, until they are completely disabled.

With respect to its development inside nematodes the fungus shows, in general, little departure from the 4 congeneric species that have previously been described and named. During the earlier stages of invasion the assimilative hyphae are often found conspicuously distended for short distances (FIG. 7, *C, b, c, e*). Apart from these swollen portions the internal mycelium is usually not clearly discernible until the animal's contents have been largely expropriated (FIG. 4, *A, C*). It is then revealed as being moderately branched and moderately beset with clamp-connections;

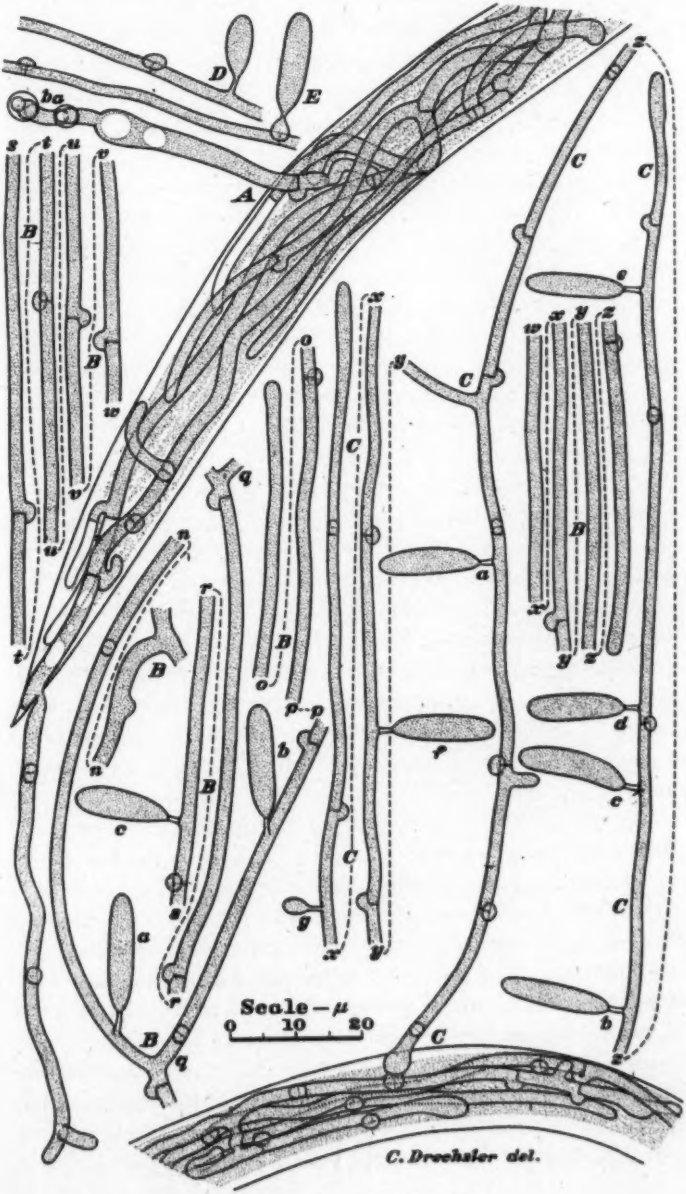

FIG. 4. *Nematoctonus haptocladus.*

many of the branches, indeed, arising from individual clamps. The assimilative hyphae, compared with those of other members of the genus, appear of intermediate coarseness,—being, on the one hand, somewhat wider than the corresponding hyphae of *Nematoctonus tylosporus, N. leiohypha,* and *N. leptosporus,* and, on the other hand, slightly narrower than the assimilative hyphae of *N. pachysporus.* At first they are filled with protoplasmic contents of rather homogeneous aspect, but as materials are withdrawn to provide for the growth externally of predaceous and conidiophorous filaments, vacuoles appear and evacuation takes place progressively until little is left within the animal's integument except empty hyphal membranes,—the remains of the eelworm being then visible only in faint outline amid the array of adhesive organs elaborated from its fleshy materials (FIG. 1, *A*). The time required to permeate the body of an eelworm after its death, and to convert its digestible contents into predaceous and conidial apparatus would seem not greatly to exceed the time taken to kill the animal after its capture. Thus, in one observed instance, a captured motionless specimen of the undescribed *Panagrolaimus,* about 325 μ long, which when first photographed (FIG. 1, *B*) showed so little internal disorganization that it could have succumbed only a little earlier, was only faintly discernible 48 hours later when it was photographed again (FIG. 5); its fleshy substance having in 2 days been completely expropriated by the fungus, and utilized in the production of approximately 30 predaceous branches and one conidium.

The paucity of reproductive apparatus, relative to the output of predaceous branches from this particular nematode, can hardly be considered unusual for the fungus. The array of approximately 34 adhesive bodies amid which the animal succumbed came from an individual nematode without the supplement even of a single conidium (FIG. 1, *A*); such failure of sporulation being rather frequent in instances where nematodes of only moderate size die in isolated positions. Where only a few conidia are produced, they usually are borne on sterigmata arising exclusively from prostrate hyphae; some of the sterigmata originating from clamp-connections (FIG. 2, *I, e, g; J, b; K.* FIG. 3, *D, k*), others from undifferentiated portions of filament (FIG. 2, *I, f.* FIG. 3, *E, a, b*). More

liberal sporulation commonly takes place where nematodes have succumbed in colony-like groups; for in addition to the scattered conidia here likewise arising from prostrate hyphae (FIG. 6, *a, b*) a larger number of conidia are produced on aerial hyphae, often several hundred microns long, which seem given over entirely to

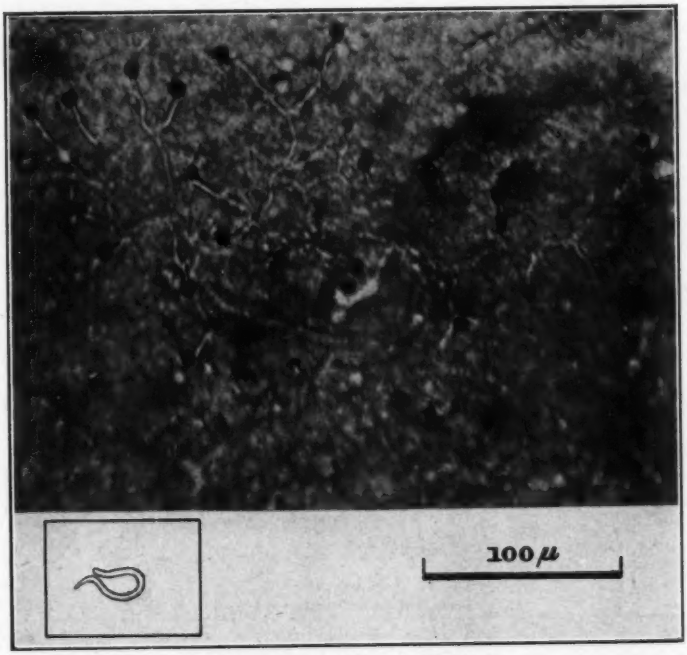

FIG. 5. *Nematoctonus haptocladus.*

asexual reproduction. These aerial hyphae (FIG. 4, *B, C*) are sometimes sparingly branched, and like the assimilative filaments are studded with clamp-connections at moderate intervals. In their earlier condition they ascend usually at a narrow angle with the horizontal. They bear their spores nearly vertically on sterigmata arising much more frequently between the clamps (FIG. 4, *B, a–c; C, a–g; D*) than directly from the clamps (FIG. 4, *E*). After their contents have largely been spent in production of conidia they decline to the substratum, much like the aerial conidiophorous hyphae of *Nematoctonus leiosporus* and *N. pachysporus.*

The sterigmata of the fungus rather closely resemble those of *Nematoctonus leiosporus* in length and distal width; but being evidently, in general, somewhat narrower at the base, they taper less markedly, and often, indeed, hardly seem to taper at all. The conidia borne on them (FIG. 2, *L, a–w.* FIG. 3, *F, a–r*) are conspicuously wider and shorter than those of *N. tylosporus* and *N.*

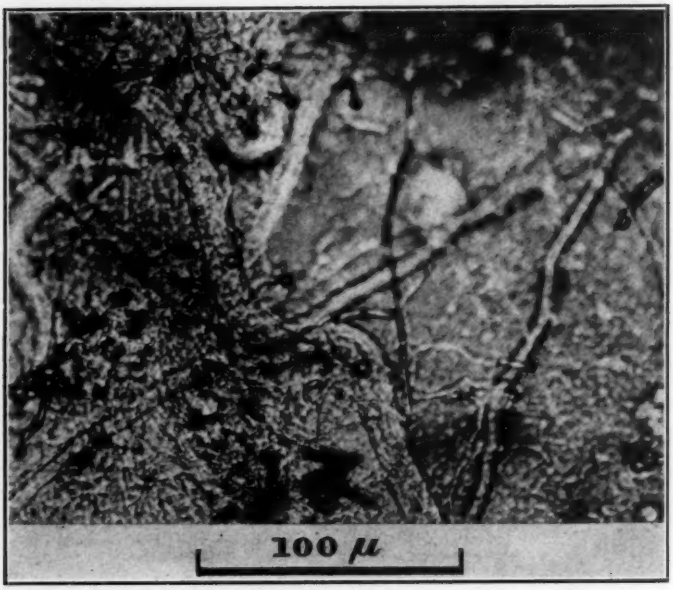

FIG. 6. *Nematoctonus haptocladus.*

leptosporus; and they have a cylindrical or elongate-ellipsoidal shape differing markedly from the tapering shape distinctive of the spores of *N. leiosporus,* as well as from the plumper strobiliform shape characteristic of the conidia of *N. pachysporus.* With respect to volume they would seem equal or very nearly equal to the conidia of *N. pachysporus,* and perhaps are slightly larger than those of *N. leiosporus;* their greater width in comparison with the latter probably more than making up for their lesser length. From the strongly curved conidia of the congeneric predaceous Hawaiian species they differ not only in their lesser length and lesser width,

but more especially in that they are straight or at most slightly curved (FIG. 2, *L, p.* FIG. 3, *F, i*).

When a conidium falls on the moist surface of an agar culture, it gives rise at its distal end to a short, erect or ascending outgrowth which terminates in a glandular cell that soon becomes surrounded by a globule of adhesive secretion (FIG. 2, *M–Q*). The material required for production of the outgrowth is provided through evacuation of protoplasmic contents from the basal portion of the

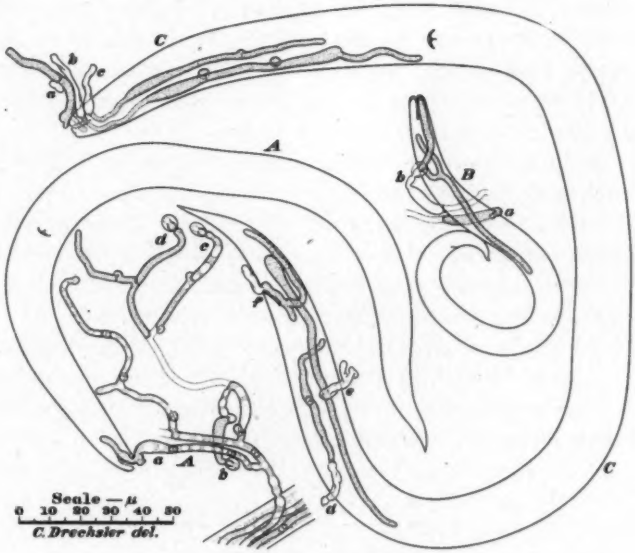

FIG. 7. *Nematoctonus haptocladus.*

spore. When this germinative development is concluded, a wall is laid down to delimit the living part from the emptied part (FIG. 2, *M–O*). In some instances, a retaining wall is laid down earlier, while evacuation is still in progress, so that in the end the spore comes to contain 2 transverse walls (FIG. 2, *P, Q*).

The adhesive outgrowth put forth by a disarticulated conidium corresponds, of course, to the erect or ascending terminations of predaceous branches; and operates quite similarly in effecting attachment to motile nematodes. Since, however, the disarticulated

spore lacks all anchorage below, and in itself is not bulky enough to impede seriously the locomotion of even a small eelworm, effective attachment of a nematode to the adhesive germ hypha results invariably in a parasitic rather than in a predaceous relationship. In fine, the conidia of the Colorado fungus are limited no less strictly to a parasitic mode of attack than the conidia of the 4 exclusively parasitic species of *Nematoctonus* previously described and named.

Infection of microscopic animals more or less directly by conidia of predaceous fungi is not unusual. Among the predaceous Zoopagaceae such infection has often been noted, for example, in the amoeba-destroying forms *Acaulopage marantica* Drechsl. (**10**: p. 144, *fig. 6, B*) *Zoöpage rhabdospora* Drechsl. (**6**: p. 375. *fig. 4, C*), and *Z. thamnospira* Drechsl. (**9**: p. 143, *fig. 2, F, G*). As the elongate conidia of these species are being carried about by the actively moving animal to whose integument they have become affixed, they intrude haustoria much like conidia of the curiously ectoparasitic *Bdellospora helicoides* Drechsl. (**4**: p. 20–26), though often revealing an essentially predaceous characteristic in continuing simultaneously to germinate by putting forth a commonplace germ hypha (**6**: p. 375, *fig. 4, C*). Sometimes minute *Amoebae* much smaller than the animals habitually serving as prey become affixed to the conidia, as also to the mycelium, of members of the Zoopagaceae,—a relevant instance being given in a figure (**5**: p. 179, *fig. 2, J*) showing a filamentous conidium of *Acaulopage macrospora* Drechsl., 52 μ long and 1.7 μ wide, from which haustoria have been intruded into 2 affixed pulvinate *Amoebae*, each about 6 μ in its greatest dimension. Naturally the very feeble *Amoebae* are easily held captive by the larger adhering conidia, which therefore can be regarded as having established a predaceous relationship even though completely lacking hyphal anchorage. Likewise conidia of the predaceous hyphomycete *Pedilospora dactylopaga* Drechsl., after directly burgeoning forth several digitiform adhesive processes (**3**: p. 396, *fig. 1, S*), have been observed capturing and destroying specimens of the small testaceous rhizopod *Geococcus vulgaris* Francé. The same rhizopod has been seen immobilized, invaded, and destroyed also by the large conidia

of the predaceous hyphomycete *Dactylella passalopaga* Drechsl. (**7**: p. 398, *fig. 1, M*).

Among the clampless predaceous hyphomycetes the manner and prevalence of attack by loose conidia varies greatly, owing in large part to the diversity of predaceous organs utilized in this group. When lying on the surface of a moist substratum the conidia of the several species employing adhesive networks sometimes germinate by giving rise exclusively to hyphal loops, or, again, sometimes produce several hyphal loops before the ordinary germ hyphae are long enough to insure good anchorage; several figures of such germination being included in Woronin's early illustrations of *Arthrobotrys oligospora* (**18**: *pl. 6, figs. 12–14*). Naturally when predaceous apparatus so weakly moored fastens on to an active nematode the whole array of loops is carried away together with the spore; the animal, nevertheless, being destined to succumb unless it should succeed in shedding the encumbering material rather promptly. At least two of the hyphomycetes that capture nematodes in constricting rings, namely *Arthrobotrys dactyloides* and *Dactylaria brochopaga*, are occasionally given to producing their remarkable predaceous organs directly on conidia (**8**: p. 483, *fig. 6, K;* p. 515, *fig. 13, L–P*) without putting forth any ordinary mycelial hyphae. A nematode caught in a predaceous ring of such origin continues to move about, carrying the spore clamped to its side, until disablement ushers in its death. When the conidium of *Dactylella asthenopaga* Drechsl. germinates, as it frequently does, by putting forth 1 or 2 predaceous organs (**8**: p. 497, *fig. 9, N, a–e*)—which in this species consist individually of a globose adhesive cell borne on a short sturdy stalk—it often adheres to specimens of *Bunonema*, and after being carried around a while, infects the sluggish animal (**8**: p. 497, *fig. 9, O*). Similar development has not been observed in *Dactylella leptospora* Drechsl., but should take place there also, since its conidia, when formed in pure culture, are frequently found bearing 1 or 2 globose cells (**8**: p. 505, *fig. 11, N, a–k*). Of all the clampless nematode-capturing hyphomycetes, *Dactylaria haptospora* Drechsl. (**11**: p. 456–461) undoubtedly displays the most consistently aggressive parasitic attack. Adaptation for such attack is clearly manifest in the curious morphology distinguishing the conidium of this

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aberrant member of the predaceous series of hyphomycetes; the adhesive terminal cell, constantly present on the spore, providing ready attachment to an animal; and the slender shape of the spore being helpful in maintaining attachment by offering little resistance to movement through substratum, and consequently affording little opportunity for removal by the scraping or shearing action attending the animal's continued locomotion.

In whatever measure disarticulated conidia of the predaceous Zoopagaceae and predaceous clampless hyphomycetes may attack animals, it yet remains true that the conidia in all predaceous species referable to these two series are capable of putting forth a mycelium extensive enough, as a rule, to afford secure anchorage against the struggles of the animals habitually serving as prey; such germination being, indeed,—except possibly for the conidia of *Dactylaria haptospora*—their more usual or preferred manner of development. However, the conidia of the clamp-bearing Colorado fungus, like those of the 4 parasitic congeners previously named, have never been seen to put forth anything but the short aerial adhesive outgrowths so obviously designed to effect attachment to roving eelworms without directly halting their locomotion. Once an adhesive outgrowth has been produced, all further extensive development appears restricted to intrusion of assimilative filaments into a animal host. Among aquatic predaceous fungi similar limitation of asexual spores to attack of animals after the usual manner of parasites, would seem present at least in *Sommerstorffia spinosa,* where according to Arnaudow's (2) original account the zoospores after rounding up a second time regularly extend a short germ tube or infection tube, and subsequently infect living rotifers through the mouth. As the empty cysts of the zoospores were reported to measure only up to 10 μ in diameter, production of germ hyphae robust enough for capture of rotifers would, of course, hardly be possible without considerable intake of nutrients. Dearth of protoplasmic content might be expected likewise to determine a parasitic manner of attack for the zoospores ascribed to *Zoophagus insidians* Somm., should these really belong to Sommerstorff's species or, for that matter, to any other aquatic rotifer-capturing phycomycete. With respect to their manner of attack it would presumably make little difference whether the

zoospores came into being through the *Pythium*-like development attributed to *Z. insidians* by Arnaudow (**1**) and Valkanov (**17**), or through the somewhat chytridial development described by Gicklhorn (**16**).

Although the clamp-bearing Colorado fungus was examined in some quantity, with the possibility of encountering basidial development being always kept in mind, it has so far been found to reproduce only by conidia. It is therefore described as a species of *Nematoctonus;* the specific name proposed for it, compounded of 2 words meaning "to fasten" and "branch," respectively, being intended to signalize its predaceous character.

Nematoctonus haptocladus sp. nov.

Hyphae assumentes incoloratae, plus minusve ramosae, plerumque 1.8–3.0 μ crassae, rarius usque 4.5 μ latescentes, in modum Hymenomycetum septato-nodosae, intra vermiculum nematoideum viventum crescentes, post mortem animalis hyphas ex magna parte procumbentes aut ascendentes extra emittentes. Hyphae procumbentes septato-nodosae, plerumque 1.8–3.0 μ crassae, rarius usque 4.5 μ latescentes, ramos plerumque 20–75 μ longos expandentes qui prope apicem abrupte in aerum se flectunt, quoque ita columellam erectam vel ascendentem 2–8 μ altam porrigente; columella corpus bilobum medio constrictum 3.5–5.5 μ longum, 2–3 μ crassum ferente; corpore bilobo mox guttula glutinosa globosa vel ellipsoidea 4–6 μ longa 3.5–5.5 μ crassa circumdato, denique saepe ad vermiculum nematoideum inhaerente, animal capiente. Hyphae ascendentes incoloratae, parvulum ramosae, mediocriter septato-nodosae, saepe circa 500 μ longae, 1.5–2.5 μ crassae. Conidia incolorata, cylindricea vel elongato-ellipsoidea, recta vel leniter curvata, sursum rotundata, deorsum saepe parve attenuata, plerumque 11–18 μ longa, 3.3–4.5 μ crassa, erecta, in apice sterigmatis vulgo 2.5–4.5 μ longi, .8–1.2 μ crassi, raro ex hyphis procumbentibus crebrius ex hyphis ascendentibus oriunda; primo continua, protoplasmatis omnino repleta, post disjunctione saepe in parte inferiore evacuata et 1–2 septis instructa, denique ex apice hypham germinationis erectam 2–8 μ altam porrigentia quae corpus glutinosum fert.

Vermiculos nematoideos diversos praecipue *Panagrolaimum* capiens consumensque etiam eadem animalia soluta necans habitat in foliis plantarum (*Cucumeris sativi, Elaeagni angustifoliae,* specierum *Syringae, Populi, Tamaricis*) putrescentibus prope Greeley, Colorado.

Assimilative hyphae colorless, more or less branched, mostly 1.8 to 3.0 μ in diameter, here and there widening to 4.5 μ, bearing clamp-connections, developing within living nematodes, after death of invaded animal putting forth prostrate and ascending external hyphae. Prostrate hyphae mostly 1.8 to 3.0 μ in diameter, here and there widening to 4.5 μ, provided with clamp-connections, ramifying at rather wide angles, the outspread branches, mostly

20 to 75 μ long, bending abruptly upward into the air to project as erect or ascending stalks 2 to 8 μ high; each stalk bearing a transversely constricted bilobate body 3.5 to 5.5 μ long and 2 to 3 μ wide; the bilobate body soon becoming surrounded by a globose or ellipsoidal droplet of glutinous substance, 4 to 6 μ long and 3.5 to 5.5 μ wide, then often adhering to a nematode and capturing it. Ascending hyphae colorless, meagerly branched, provided moderately with clamp-connections, often about 500 μ long and 1.5 to 2.5 μ wide. Conidia colorless, cylindrical or elongate-ellipsoidal, straight or slightly curved, broadly rounded at the tip, often tapering slightly toward the base, measuring mostly 11 to 18 μ in length and 3.3 to 4.5 μ in greatest width, borne erect on sterigmata, commonly 2.5 to 4.5 μ long and .8 to 1.2 μ wide, that arise sparingly from prostrate hyphae and in closer arrangement from ascending hyphae; the spores at first continuous and filled with protoplasm throughout, but after disarticulation regularly becoming evacuated proximally and partitioned proximally by 1 or 2 septa in extending from the apex an erect or ascending germ hypha, mostly 2 to 8 μ long, whereon an adhesive body is borne terminally.

Capturing and consuming various nematodes (especially *Panagrolaimus* sp.) and also destroying these animals in free condition, it occurs in decaying plant (*Cucumis sativus, Elaeagnus angustifolia, Syringa* sp., *Populus* sp., *Tamarix* sp.) leaves near Greeley, Colorado.

As was set forth in a recent paper (**15**: p. 3) elaboration of adhesive material in easily visible masses previous to encounter with prey has not been noted among the predaceous Zoopagaceae, and among the clampless predaceous hyphomycetes has been noted only in *Arthrobotrys entomopaga* Drechsl., a species primarily adapted for capture of springtails, though occasionally also capturing nematodes (**14**). The statement then made to the effect that no fungus specially adapted for capture of eelworms secretes adhesive material in visible quantities beforehand (**15**: p. 3, lines 9–13), now requires modification limiting its application to the 2 main predaceous groups. Indeed, the anticipative secretory behavior wherein *Nematoctonus haptocladus* differs so markedly from the many nematode-capturing fungi taxonomically alien to it, is present likewise in the congeneric predaceous Hawaiian species which "utilizes erect hyphal processes bearing droplets of adhesive mucus at the tip" (**12**: p. 780, lines 33–35).

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EXPLANATION OF FIGURES

FIG. 1. *Nematoctonus haptocladus;* adhesive bodies, about 34 in number, borne aloft on the upcurved tips of prostrate branches coming from the assimilative mycelium in a nematode, *A* (*Panagrolaimus* sp.), that has become nearly invisible owing to expropriation of contents. A second nematode, *B*, of the same species is shown attached to two of the adhesive bodies. Unretouched photomicrograph, × 300.

FIG. 2. *Nematoctonus haptocladus;* drawn to a uniform magnification with the aid of a camera lucida, from a moist preparation covered with a cover glass; × 1000 throughout. *A*, Branching hyphae extended under the surface of an agar culture from a specimen of *Panagrolaimus* sp. that had succumbed in a submerged position. *B*, Ramifying prostrate hyphal system with 5 predaceous branches bearing aloft the adhesive bodies *a–e*; the ramifying system having developed terminally on a hypha that came from a submerged eelworm to the surface of the culture. *C*, Terminal portion of a prostrate hypha with a single predaceous branch bearing aloft the adhesive body *a*. *D*, Portion of prostrate mycelium with a predaceous branch holding aloft the adhesive body *a*; one of the 3 clamp-connections present is shown empty of contents and only faintly visible. *E*, *F*, Predaceous branches, each bearing aloft an adhesive body, *a*; through evacuation of protoplasm, the living part of each branch has been reduced to a structure not differing much in shape, size, and function from a conidium that has put forth an adhesive germ hypha. *G*, Predaceous branch arising directly from an assimilative hypha, and terminating in the adhesive body *a*. *H*, Predaceous branch that has given rise successively to 3 adhesive bodies, *a*, *b*, *c*. *I*, Prostrate ramifying filament with 4 predaceous branches bearing aloft the 4 adhesive bodies *a–d*; a young conidium, *e*, is found arising from a clamp-connection, and 2 fully formed conidia, *f*, *g*, are shown attached, the latter arising from a clamp. *J*, Prostrate branch, in large part evacuated, terminating in an adhesive body, *a*, and bearing a conidium, *b*, on a sterigma arising from a clamp. *K*, Conidium borne on an empty sterigma arising from a clamp. *L*, Newly detached conidia, *a–w*, showing variations in size and shape. *M–O*, Disarticulated conidia, each of which has become evacuated proximally in putting forth a germ hypha with an adhesive body. *P*, *Q*, Detached conidia of similar development, but in which 2 septa were laid down during evacuation of the proximal part.

FIG. 3. *Nematoctonus haptocladus;* drawn to a uniform magnification with the aid of a camera lucida, from a moist preparation covered with a cover glass; × 1000 throughout. *A*, Portion of body of a nematode, referable to *Panagrolaimus* sp., that is occupied by assimilative mycelium; one of the assimilative filaments having put forth a prostrate ramifying system with 6 predaceous branches, whereon are borne 7 adhesive bodies, *a–g*. *B*, Prostrate ramifying system with 2 predaceous branches that after producing the adhesive bodies, *a* and *b*, respectively, have continued growth. *C*, Portion of prostrate ramifying system with a predaceous branch terminating in the adhesive body *a*. *D*, Somewhat extensive prostrate ramifying system, whose predaceous branches bear aloft the 10 adhesive bodies *a–j;* a conidium, *k*, is shown attached to a sterigma arising from a clamp-connection. *E*, Portion

of prostrate hyphal system showing 2 conidia, *a* and *b*, borne on empty sterigmata. *F*, Newly disarticulated conidia, *a–r*, showing variations in size and shape.

FIG. 4. *Nematoctonus haptocladus;* drawn to a uniform magnification with the aid of a camera lucida, from a moist preparation covered with a cover glass; × 1000 throughout. *A*, Posterior portion of a dead specimen of *Panagrolaimus* sp. occupied by assimilative mycelium of which terminal parts have become evacuated in supplying material for production of external hyphae; one of the 2 external hyphae shown has produced 2 adhesive bodies, *a* and *b*. *B*, Branched ascending hypha bearing 3 conidia, *a–c;* owing to lack of space the hypha is shown in parts whose proper continuity is indicated by means of the alphabetical sequence *n–s*; *n–q* being a proximal element that bears distally the branches *q–o* and *q–s*. *C*, Portion of specimen of *Panagrolaimus* sp. occupied by assimilative filaments, one of which has put forth an ascending branched hypha whereon are borne 6 mature conidia, *a–f*, and a very young, growing conidium, *g*; from lack of space the hypha is shown in parts whose proper continuity is indicated by the letters *x*, *y*, *z*. *D*, Portion of ascending filament showing a conidium in half-grown state. *E*, Portion of ascending filament showing a conidium almost full-grown but still continuous with the sterigma; the latter arising from a clamp-connection.

FIG. 5. *Nematoctonus haptocladus;* external growth made in 48 hours from the same individual eelworm shown in figure 1, *B*; approximately 30 predaceous branches, each with an adhesive body, being recognizable; a single conidium, also present, not being recognizable; unretouched photomicrograph, × 300. After the photograph shown in figure 1 had been taken the culture was flooded with water for a few minutes, completely obliterating all the prostrate hyphae and adhesive bodies derived from nematode *A* of that figure; so that all the prostrate hyphae and adhesive bodies visible in the later photograph were necessarily produced during the intervening 2 days.

FIG. 6. *Nematoctonus haptocladus;* portion of colony-like group of nematodes mostly referable to *Panagrolaimus* sp.; showing, in addition to scattered predaceous branches, 2 conidia, *a* and *b*, arising from prostrate filaments; unretouched photomicrograph, × 400.

FIG. 7. *Nematoctonus haptocladus;* drawn to a uniform magnification with the aid of a camera lucida, from a moist preparation covered with a cover glass; × 500 throughout. *A*, Prostrate ramifying hyphal system with 4 adhesive branches, *a–d*, on one of which, *a*, a specimen of *Panagrolaimus* sp. has been captured; slight movement of the animal from time to time showed it was still alive, though invaded by an assimilative hypha over 10 μ long. *B*, Small specimen of *Paraphelenchus pseudoparietinus* 48 hours after being captured by adhesion to 2 predaceous branches, *a* and *b*; both predaceous branches are still attached to the parent hypha, though their mycelial connection has become indiscernible owing to evacuation of intercalary hyphal parts. *C*, Specimen of *Panagrolaimus* sp. still capable of slight movement, but manifestly close to death, owing to hyphal invasion from 6 adhering predaceous branches, *a–f*; all the predaceous branches had been torn from their attachments to mycelium, and the animal was succumbing in a free condition.

VARIABILITY OF PYTHIUM ULTIMUM FROM GUAYULE[1]

W. A. Campbell and Bailey Sleeth

(with 2 figures)

INTRODUCTION

In the course of investigations on damping-off and root rot of guayule (*Parthenium argentatum* Gray) in the nurseries near Salinas, California, a *Pythium* was isolated frequently and consistently from diseased material. These isolates were similar in mat characters and growth rate. However, microscopic examination of the mats when grown on cornmeal agar revealed that a relatively large number of them failed to produce oogonia and antheridia upon which the identification of *Pythium* largely depends but produced only globose sporangia or sporangia-like bodies. Those that produced oogonia and antheridia were identified as *Pythium ultimum* Trow.[2] In the disposition of the non-oospore-producing isolates the writers (1) have indicated that they should also be considered as *P. ultimum* on the basis of their association with oospore-producing isolates.

The assignment of these non-oospore-producing isolates to *Pythium ultimum* raised certain taxonomic questions concerning variability within the species, particularly whether or not the existence of oospore- and non-oospore-producing forms could result from factors other than evironmental. Therefore, the purpose of this paper is not only to report on the variability observed

[1] The interest and suggestions of the following individuals during the course of this study or in the preparation of the manuscript are gratefully acknowledged: H. N. Hansen, W. C. Snyder, John T. Middleton and Gladys Baker.

[2] The interpretation of Middleton (8) in respect to *Pythium ultimum* has been followed in this study. However, it is apparent from the variability observed in the structure of the antheridium and manner of attachment to the oogonium that Van Luijk's (12) contention that *P. ultimum* and *P. Debaryanum* belong to a single species in which considerable variation may be recognized deserves further investigation.

in spore formation (oospores and sporangia) in a number of *P. ultimum* isolates, growth rate in culture, pathogenicity to emerging guayule seedlings but also the results of successive hyphal-tip and single-spore subculturing of several isolates.

CLASSIFICATION OF ISOLATES

The majority of the isolates used in the present study were obtained over a period of two years by plating diseased guayule tissue on Sleeth's agar medium (**10**). One hundred and twenty-one isolates, which included 51 previously reported by the writers (**1**), were grown simultaneously in Petri dishes on Difco cornmeal agar in diffused light at room temperatures ranging from 20 to 28° C.[3] After 14 days the cultures were examined microscopically and classified on the basis of relative abundance of oospores and sporangia.[4]

The isolates varied greatly in the number of oospores and sporangia produced in culture and in the ratio between the two spore forms. Three classes or types of isolates were recognized and for convenience were designated as O, OS and S (FIG. 1 and TABLE 3). The O type isolates produced mainly oospores and relatively few large globose terminal sporangia. However, small intercalary chlamydospores were commonly present as were disorganized oogonia. The OS type isolates produced oospores and a variable number of terminal and intercalary sporangia. The S type isolates produced only sporangia in culture. These varied from few to many and were characteristically terminal, globose, and up to 32 μ in diameter.

The OS type isolates were the most numerous comprising 71 of the 121 isolates examined; the S type was next with 47 isolates; and the O type isolates were relatively few with only 3 so classified.

[3] All results unless otherwise specified herein reported on spore production are based upon observations made on Difco cornmeal agar, following the procedure given above.

[4] Although the structures analogous to sporangia in *Pythium ultimum* do not produce zoospores but germinate with a germ tube, and have been termed chlamydospores or conidia, the term sporangia will be used rather than chlamydospores or conidia to designate all large globose non-oospore-producing structures in order to avoid confusion in type designations with those established by Hansen (**3**) in his studies on the dual phenomenon in imperfect fungi.

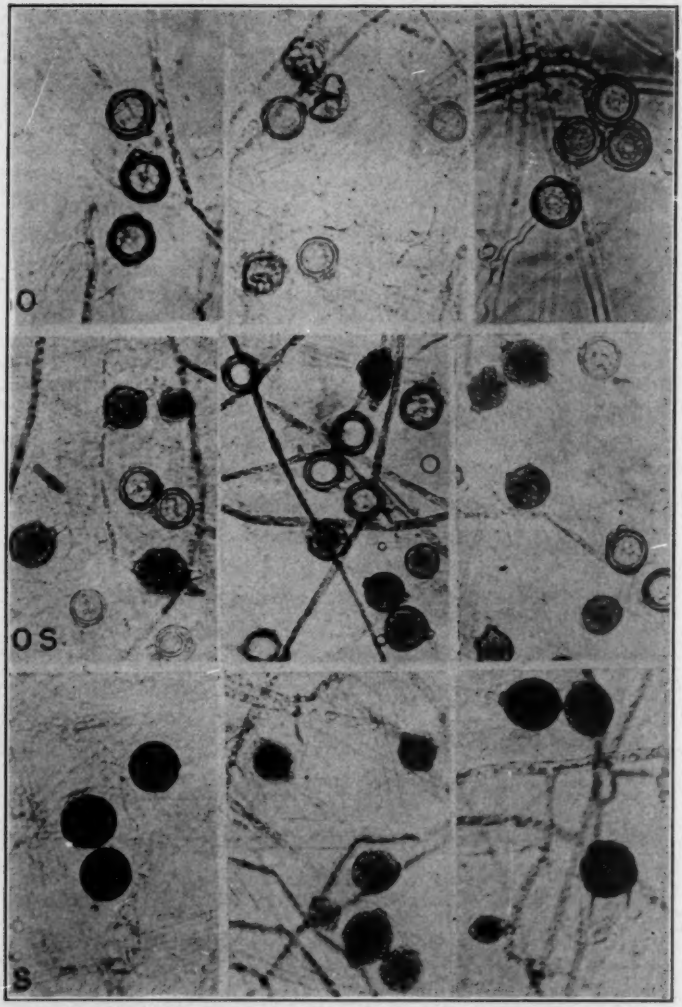

FIG. 1. Types of *Pythium ultimum* isolates based upon relative numbers of oospores and sporangia produced on cornmeal agar.

Top—Type O isolates in which oospores or disorganized oogonia are predominant. Sporangia may occur but these are relatively few in proportion to oospores.

Center—Type OS isolates in which both oospores and sporangia are prevalent. The number of oospores in proportion to sporangia may be quite variable in this class.

Bottom—Type S isolates in which only sporangia develop.

HYPHAL TIP SUBCULTURES OF 25 ORIGINAL ISOLATES

The original *Pythium* isolates were obtained from small bits of diseased tissue and the possibility existed that some of them were mixtures of two or more types. In order to eliminate the possibility of mixed cultures, single hyphal-tip transfers were made from 25 of the 121 *Pythium* isolates which exhibited the widest range of variation in spore formation; three were O type isolates; 15 OS and 7 S (TABLE 1).

TABLE 1

DATA ON *Pythium ultimum* ISOLATES USED IN VARIABILITY STUDY
All isolates were obtained from guayule in the vicinity of Salinas, California.

Isolate	Culture type	Material from which obtained	Location	Date isolated
C111	O	Pink root rot lesion	Nursery	9/ 2/44
912	O	Seedling root rot	Field seeding	7/24/44
945	O	Pink root rot lesion	Nursery	9/12/44
B17	OS	Seedling root rot	Nursery	6/12/43
T52[1]	OS	Pink root rot lesion	Field seeding	8/12/43
C104[1]	OS	Pink root rot lesion	Nursery	9/ 1/44
515	OS	Seedling root rot	Field seeding	7/ 6/43
690	OS	Seedling root rot	Nursery	5/10/44
700	OS	Seedling root rot	Nursery	5/12/44
736	OS	Seedling root rot	Nursery	5/16/44
740	OS	Seedling root rot	Nursery	5/17/44
800	OS	Root rot	Nursery	6/ 2/44
917	OS	Seedling root rot	Field seeding	7/24/44
968	OS	Pink root rot lesion	Field seeding	9/21/44
980	OS	Pink root rot lesion	Field seeding	9/21/44
1010	OS	Seedling root rot in moisture experiment	Greenhouse	12/23/44
1041	OS	Damped-off seedlings	Greenhouse	12/26/44
1046	OS	Damped-off seedlings	Greenhouse	12/26/44
B25	S	Seedling root rot	Nursery	6/ 8/43
B48a	S	Seedling root rot	Nursery	6/ 9/43
50a	S	Seedling root rot	Nursery	6/ 9/43
T53	S	Seedling root rot	Field seeding	8/12/43
648	S	Seedling root rot	Nursery	4/28/44
1042	S	Damped-off seedling	Greenhouse	12/26/44
1050	S	Damped-off seedling	Greenhouse	12/26/44

[1] Identification verified by John T. Middleton.

Twenty hyphal-tip subcultures were made of each of the 25 isolates and examined for the presence of oospores and sporangia after 14 days on cornmeal agar. The hyphal-tip subcultures from all of the O and S type isolates were similar to the parent cultures. Four of the OS type isolates yielded subcultures of both O and S

or OS and S types indicating the possibility that they had been carried in the laboratory as mixed cultures (TABLE 2).

TABLE 2

HYPHAL-TIP SUB-ISOLATES OBTAINED FROM 4 *Pythium ultimum* ISOLATES WHICH WERE MIXED OR UNSTABLE AS TO TYPE

Isolate	Original isolate	Hyphal-tip cultures		
		Type O	Type OS	Type S
	Type	*Number*	*Number*	*Number*
C104	OS	9	0	11
736	OS	1	0	19
800	OS	0	11	9
980	OS	0	9	10

VARIATION IN SPORE NUMBERS

In order to determine the relative abundance of oospores and sporangia and to verify the classification of the 25 selections (TABLE 1), 26 hyphal-tip subcultures which included one or several from most of the 25 selections were grown in duplicate on corn-meal agar. The plates were incubated in diffused light, at room temperature for 17 days. Spore counts were made with the high power of the microscope (10X ocular and 4 mm. objective). Forty observations were made, 20 on each plate, by two observers and the average number of oospores and sporangia computed (TABLE 3). Only fully developed oospores were counted; immature or disorganized oogonia were disregarded. Small intercalary or terminal chlamydospores were not counted and sporangia were considered as the large terminal bodies ranging in size from approximately 20 to 32 μ.

The data (TABLE 3) on relative spore numbers show considerable variation in the numbers of spores of each kind produced by the different isolates. The line of separation between the different types is likewise not sharp and distinct and there are isolates that fall on the borderline between the O and OS types and between OS and S types. This is to be expected and does not necessarily invalidate the convenience afforded by classification of the isolates of *Pythium ultimum* into types.

Hartley (5) in dealing with a *Pythium* species which he designated as *Pythium Debaryanum* observed a similar condition in respect to variability in spore formation. He reported strains with few oospores and many chlamydospores (sporangia), those in which a reverse relationship existed and also intimated that certain strains produced few if any oospores.

TABLE 3

RELATIVE ABUNDANCE OF OOSPORES AND SPORANGIA FOR DIFFERENT ISOLATES OF *Pythium ultimum* WHEN GROWN ON CORNMEAL AGAR AT ROOM TEMPERATURE FOR 17 DAYS

Culture	Type	Average per field [1]		Culture	Type	Average per field	
		Oospores	Sporangia			Oospores	Sporangia
945-14	O	2.9	.01	690-1	OS	3.3	4.2
912-1	O	2.5	.2	980-1	OS	2.4	4.2
C111-1	O	2.8	.3	700-1	OS	3.1	6.3
736-14	O	1.1	.9	980-14	OS	0.1	8.0
B17-1	OS	3.6	1.1	648-1	S	0	2.0
800-1	OS	5.1	1.2	B25-1	S	0	2.1
515-1	OS	4.8	1.5	C104-9	S	0	2.5
917-1	OS	2.9	1.6	1050-1	S	0	2.5
1010-1	OS	5.0	2.0	T53-1	S	0	2.6
740-1	OS	4.2	2.0	1042-2	S	0	3.2
1046-1	OS	2.3	3.0	50a-1	S	0	4.0
968-1	OS	6.0	3.0	B48a-1	S	0	4.2
T52-1	OS	3.5	3.6	980-17	S	0	7.0

[1] Average per high power field (10X ocular × 4 mm. objective) of 40 observations on 2 plates of each isolate.

SPORE FORMATION ON SPECIAL MEDIA

Various workers with *Pythium* have experienced difficulty in inducing certain isolates to produce oospores on standard culture media. For example, Rands and Dopp (9) found that many but not all isolates of *P. arrhenomanes* which failed to produce oospores on ordinary culture media did so if humic extract was added. Johann (6) reported that grated carrot agar was helpful in securing oospores of several species which did not readily produce them on ordinary agar media. Following the suggestions of these and other workers the *Pythium* isolates that failed to produce oospores on cornmeal agar were grown on water agar, cornmeal agar plus an extract of guayule shrub, cornmeal agar plus soil extract, and

agar made from freshly grated carrots. None of these special agars stimulated the formation of oospores in the S type cultures.

Cornmeal agar appears to be the most satisfactory culture medium for the production of oospores in *Pythium ultimum*. Ordinarily maximum spore production is achieved at room temperature in 14 days and little or no increase in spore numbers could be noted on further aging. Thickness of agar influences sporangia production and usually more sporangia are produced on thick agar layers than on thin. The presence or absence of oospores in *P. ultimum* appears to be a fairly stable quality which is not readily affected by the quantity of culture medium.

GROWTH AT CONSTANT TEMPERATURES

Pythium ultimum isolates of the three different types appeared to have the same growth rate when grown at room temperatures ranging from 20° to 28° C. In order to ascertain if this were also true at higher and lower temperatures, 29 hyphal-tip subcultures which included 5 type O, 13 type OS and 11 type S were grown on cornmeal agar at constant temperatures of 10, 15, 20, 25, 30, 35 and 40° C.

Inoculations were made from 2- to 4-day-old cultures, growing on cornmeal agar, using a uniform circular piece of inoculum 4 mm. in diameter. The inoculum was placed mycelium side down at the edge of the agar in the Petri plate so that the mycelium would have approximately 8 cm. of agar for growth before reaching the opposite side of the dish. The plates were held at room temperature from 4 to 5 hours until discernible growth had started, then inverted and incubated for 48 hours at the desired temperature in incubators which maintained these temperatures within 1° C.

The growth rates of all 29 hyphal-tip subcultures were remarkably similar regardless of type and agreed closely with the temperature growth data for *Pythium ultimum* presented by Middleton (**8**). There appeared to be no noticeable difference between the rate or kind of growth of the S type cultures and those of O and OS types. The optimum temperature for mycelial growth of *P. ultimum* as determined by these isolates falls between 25 and 30° C.

PATHOGENICITY

Pathogenicity tests were made on emerging guayule seedlings with the 26 single hyphal-tip subcultures listed in table 3. The different subcultures were grown on a wheat bran mixture (wheat bran 400 grams, dextrose 20 grams, distilled water 600 ml.) which had been sterilized by autoclaving for 60 minutes at 15 lbs. pressure. After 10 days of growth at room temperature in which time the mycelium had permeated the 40 grams of bran mixture in a 250 ml. Erlenmeyer flask, the contents of 2 flasks of the same isolate were uniformly distributed over the surface of pasteurized soil in eight 6-inch clay pots. The layer of inoculum was then covered with ½ inch of soil upon which 50 guayule seeds were sown and covered with ¼ inch of pasteurized sand. The eight pots inoculated with a single isolate of *P. ultimum* were randomized in 8 blocks on benches in the greenhouse. The pots were watered and cared for so as to secure maximum emergence in the checks. All of the 26 isolates were tested twice for pathogenicity, the second test being made approximately one month after the first.

Seedling emergence counts were made daily from the day the first emergence occurred, to 15 days after sowing. Usually the peak of emergence had occurred by the 10th day. The seedling emergence in the checks, which included pots with and without sterilized bran inoculum, ranged from 90 to 95 per cent of the germinable seed sown. The average emergence for any one isolate in the fungal inoculated pots never exceeded 2 per cent of the germinable seed sown.

All of the isolates were pathogenic to guayule germination and emergence and of comparable virulence. Thus there was no indication that virulence of the isolates tested was associated with the presence or absence of oospores.

VARIATION IN HYPHAL TIP AND SINGLE SPORE SUBCULTURES OF SELECTED ISOLATES

The occurrence of the O, OS and S types which were isolated from diseased guayule seedlings was highly suggestive of the "dual phenomenon" of Hansen (3) which is common in the imperfect fungi. In order to determine whether or not the differentiation

into oospore, oospore-sporangia, and sporangial types of *Pythium ultimum* isolates could be explained on the basis of the dual phenomenon, extensive hyphal-tip and single-spore subcultures were made of selected hyphal-tip isolates. In obtaining single-spore cultures it was found that the oospores, except rarely, failed to germinate on solid agar medium and that it was necessary to use the asexual spores for this purpose. The latter germinated readily in dilution plates on Sleeth's medium or water agar within 3 to 4 hours at room temperature. Their relatively large size, from 20 to 32 μ in diameter, made it a simple operation to lift the germinating spores from the agar disk under the binocular dissecting microscope. Hyphal-tip subcultures were made 24 hours after inoculating on Sleeth's medium. This medium was more suitable for this purpose than water or cornmeal agars because the hyphae tended to separate better and it was easier to remove the individual hyphal tips. A hyphal tip was cut with a sharp flattened needle and removed from its position to an area where no hyphae had penetrated. After checking with the binocular microscope to see that only one tip was included in the agar piece, it was transferred to a Petri dish or test tube.

Six isolates were selected for hyphal-tip or single-spore subculturing. Two, 1041 and 1046, were chosen because they produced approximately equal numbers of oospores and sporangia in culture and had been pure as to type when isolated; two, 1042 and 1050, were chosen because they produced only sporangia in culture and had been isolated as pure types; and two, C104 and 980, were selected because they had been isolated originally as mixed types or had separated into types O and S for the former and OS and S for the latter on subculturing (TABLE 2).

Three generations of single-spore cultures of 1041 and 1046 failed to produce a single subculture which differed noticeably from the OS type isolates from which they were derived. The number of single-spore cultures in the three generations for 1041 was 63, 44 and 50 respectively, and for 1046, 85, 68 and 24.

One generation of single-spore subcultures of 1042 and 1050 failed to produce a single one with oospores. Seventy-eight single-spore cultures were made from 1042 and 112 from 1050. No further single-spore subcultures were made of these isolates as the

large number involved in the first generation was considered sufficient to detect any variation toward oospore production that might exist.

Isolate C104, obtained in September 1944, had been carried in the laboratory for approximately 6 months at the time hyphal-tip subcultures were made. One mass mycelial transfer of the original isolate was stored in the refrigerator and another was left at room temperature in the laboratory. In preparation for single hyphal-tip subculturing mycelial transfers were made from the two cultures of C104 which were stored under different conditions. The transfer from the culture stored in the refrigerator produced both oospores and sporangia; the one from the culture kept at room temperature produced only sporangia. Since this was the first time that a change had been noted in type of spore production, 3 successive mass mycelial transfers were made of the S type culture and carefully examined for oospores. None was found. The OS type culture of C104 segregated into 9 type O and 11 type S on hyphal-tip subculturing. Further hyphal-tip subculturing of a type O subculture obtained by the first hyphal-tip series gave all type O cultures; a type S subculture likewise gave all S types. The S type culture which originated from a mass mycelial transfer was also hyphal tipped yielding 3 type O subcultures and 17 type S.

The original OS type isolate 980 segregated into 9 OS type subcultures and 10 S type on hyphal tipping (TABLE 2). The 9 OS type subcultures were different from those obtained from any other *Pythium ultimum* isolate in that they varied considerably in the number of oospores produced. Two OS type subcultures, selections 1 and 14, were saved for further hyphal tipping as was one S type, selection 17. Oospores were fairly common in selection 1 (TABLE 3), but extremely rare in selection 14, and entirely lacking in selection 17.

Seventeen hyphal-tip transfers of selection 1 segregated into 3 type OS and 14 type S (FIG. 2). Eight OS type and 17 S type subcultures were obtained from single-spore transfers.

Twenty hyphal-tip transfers of selection 14 segregated into 1 type O (selection 13), 2 OS type (selections 11 and 12) and 17

S type. Twenty-five single-spore subcultures of selection 14 gave 6 OS type and 19 S type.

A third hyphal-tip generation using the type O subculture selection 13, as the base, gave 16 or all O type cultures. A third hyphal tipping of the type OS subculture selection 11, gave 14 OS

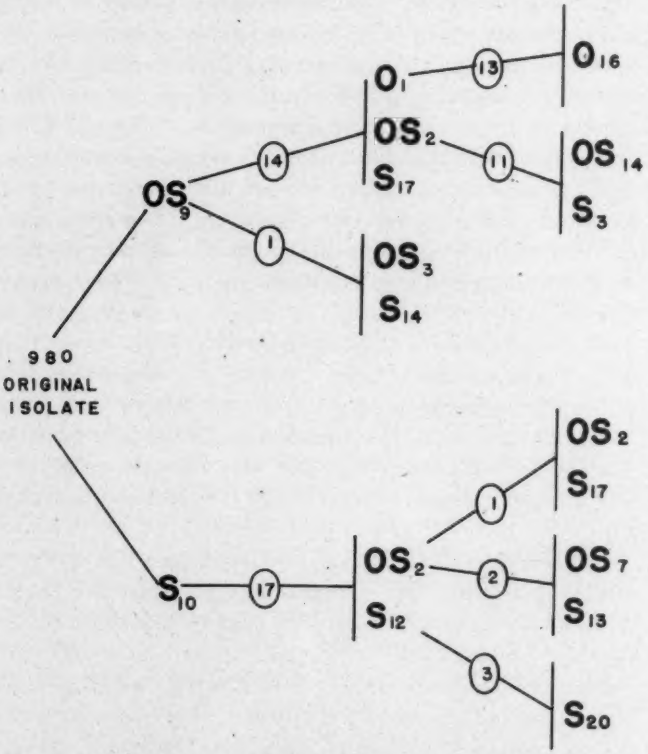

FIG. 2. Culture Types Obtained by 3 Successive Hyphal Tippings of Isolate 980.

type and 3 S type cultures. These 14 OS type subcultures exhibited considerable variation in respect to the number of oospores produced. In 3 of the subcultures only one or two oospores could be found, in 4 a few oospores were present so that one or more could be found in each low power field, and in 7 many oospores were produced and correspondingly fewer sporangia.

The second generation hyphal tipping of the type S subculture designated as selection 17 gave 2 OS type and 12 S type isolates (FIG. 2). Selection 1 from the above generation produced very few oospores, in fact, only one could be located on thorough examination on the plate. Selection 2 of the same generation, also an OS type culture, produced a number of oospores. These 2 selections were hyphal tipped for the third generation. Selection 1 gave 2 cultures that were OS type and 17 that were S type. Selection 2, which produced more oospores, gave 7 type OS and 13 type S cultures. A third generation hyphal tipping of selection 3 in which no oospores were found gave 20 type S subcultures.

There was some indication that sectoring leading to variation in spore formation occurred in culture even though no apparent change in mycelial growth could be detected. In making hyphal-tip transfers from a rapidly growing 24-hour-old culture, 40 to 50 mm. in diameter, individual hyphal tips were picked off successively in a clockwise manner and serially numbered. In several cases 2 to 4 hyphal-tip subcultures were obtained from branched hyphae of a single hyphal strand and on two different occasions 2 or more successive hyphal-tip subcultures were found to be of a different type from the others. For example, the 3 subcultures that produced oospores in selection 14 (FIG. 2) developed from hyphal tips 11, 12 and 13. Likewise the two OS type subcultures from selection 17 developed from hyphal tips 1 and 2.

DISCUSSION

The investigation of variability of *Pythium ultimum* was the outgrowth of what disposition should be made of isolates which in all essentials, except the lack of oospores, were comparable to ordinary isolates of *P. ultimum*. In these isolates only the asexual spore stage, sporangia (or chlamydospores), was present. According to various keys for the determination of *Pythium* species. isolates which fail to produce oospores are difficult to classify (**7**, **8**). Similar conditions have been reported by various investigators (**5**, **9**, **11**) and have been disposed of by considering such non-conforming isolates to be of the same species as that which was most frequently isolated from the suscept and which produced oospores. A similar position was taken by the writers in an

earlier paper (1). This disposition of the non-oospore-producing isolates of *Pythium ultimum* was justified by finding that hyphal-tip subcultures of the OS type gave rise to the S type by means of successive hyphal-tip or single-spore transfers.

The present study has demonstrated that the species *Pythium ultimum* is composed of a number of strains which differ in their capacity to produce either oospores or sporangia or both. Furthermore, the evidence presented shows that the relative numbers of oospores and sporangia produced by a strain were fairly constant. That is, a strain which produced many oospores and few sporangia, one that produced few oospores and numerous sporangia, or one that produced only sporangia, would likely continue to do so for an indefinite number of hyphal-tip or single-spore transfers.

The stability of many *Pythium ultimum* strains and the difficulty of securing divergent lines from hyphal-tip or single-spore transfers may be explained by the homothallic nature of the fungus (8, 13) and the failure of the mycelium to anastomose. No anastomosis of hyphae was observed in any of the *P. ultimum* cultures examined. These two conditions tend to fix the characteristic of a strain and reduce to a minimum the chances for variation. Even though numerous strains of *P. ultimum* are found in a given locality it is believed to be the result of strain stability rather than a tendency to readily give rise to different strains.

The stability of single-spore or single hyphal-tip isolates in culture and their seeming reluctance to "break-down" into divergent types is assumed to be evidence that the various strains are fairly stable in nature. On the other hand it is reasonable to expect certain strains to be less stable than others, and a very small number to be exceedingly unstable. The behavior of the single hyphal-tip and single-spore isolates obtained in culture substantiates this assumption. Although, most of the isolates in successive single-spore or hyphal-tip transfers were similar to the parent culture, some few and particularly those from isolate 980, an oospore-sporangia type, gave rise to the parent type and both the S and O types.

To account for variation within a fungus species many suggestions and possible explanations may be found in the literature. Tompkins et al. (11) found that certain isolates of *P. ultimum*

from pumpkin and watermelon failed to produce oospores in culture. They stated "It seems probable that the failure of certain isolates to develop oospores is the result of failure to form antheridia. Since there is no evidence that a heterothallic condition occurs in the species it is suggested the monosexuality exhibited by these strains may be genetic in nature." Hansen (3) stated that variability in certain fungi "is not due to mutations (genetic instability) in pure cultures but rather to the fact that many fungi as they exist in nature, although operating as definite entities, are composed of two distinct elements or individuals." He applied the term "dual phenomenon" to this condition and indicated that it is "due to a condition of heterocaryosis." Contrary to this view Chilton (2) in a report on a number of variants of *Colletotrichum destructivum* stated that the "variants were not due to a heterocaryotic condition of the mycelium," but that "the evidence indicates they are genetic entities differing from the cultures from which they arose." He therefore suggested that they are mutants. Hansen (4) in further discussions on variability stated that both heterocaryosis (dual phenomenon) and genetic instability (mutation) operate in creating variability in fungi. In a homothallic fungus, such as *P. ultimum*, in which the sexual phase is seemingly of minor importance in propagating the fungus, it is suggested that variation may develop as the result of mutation (gene mutation or somatic segregation) which gives rise to a heterocaryotic condition. Somatic mutation would likely occur as readily in a homocaryotic individual as in a heterocaryotic one and it might well be that this sort of mutation occurs more frequently than is suspected.

SUMMARY

One hundred and twenty-one isolates of *Pythium ultimum* Trow from diseased guayule (*Parthenium argentatum* Gray) seedlings were classified into 3 types on the basis of relative abundance of oospores and sporangia (chlamydospores) when grown on Difco cornmeal agar. Three of the isolates which produced mainly oospores were classified as type O; 71 which produced oospores and few or many sporangia were classified as type OS; and 47 which produced only sporangia were classified as type S.

Twenty hyphal-tip subcultures were taken from each of 25 selected isolates. The subcultures from three O type, 11 OS type and 7 S type were similar to the parent cultures; those from 4 OS type isolates segregated into two that gave type O and type S subcultures and 2 that gave type OS and type S indicating that these four isolates were mixed cultures or were unstable as to type.

In an effort to induce isolates of the S type (non-oospore formers) to produce oospores they were grown on cornmeal agar, water agar, cornmeal agar plus an extract of guayule shrub, cornmeal agar plus an extract from well-decayed leaf mold, cornmeal agar plus soil extract, and agar made from freshly grated carrots. None of these media stimulated the formation of oospores in the S type isolates.

Five O type, 13 OS type and 11 S type hyphal-tip subcultures of 25 isolates were found to have very similar rates of growth on cornmeal agar at each of the following constant temperatures: 10°, 15°, 20°, 25°, 30°, 35° and 40° C. The growth rate at these temperatures was comparable to that reported (8) for *Pythium ultimum*. The optimum temperature for mycelial growth was found to be between 25° and 30° C.

Twenty-six hyphal-tip subcultures which included four O type, 13 OS type and 9 S type were tested for pathogenicity on emerging guayule seedlings in the greenhouse. All of the different types were found to be pathogenic and of comparable virulence in causing preemergence loss to guayule seedlings.

In general most hyphal-tip or single-spore subcultures of *Pythium ultimum* isolates were stable as to type. However, one isolate, 980, readily gave rise to other types. Successive hyphal-tip transfers of a hyphal-tip subculture of 980 of the OS type gave rise to both the O and S types as well as the parent type. Three generations of single-spore (sporangium) subcultures from two isolates of the OS type were all OS type similar to the parent. Likewise one single-spore generation of two S type isolates were all of the S type.

SPECIAL GUAYULE RESEARCH PROJECT,
SALINAS, CALIFORNIA

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GUIGNARDIA RHODORAE, THE PERFECT STAGE OF PHYLLOSTICTA MAXIMA ON RHODODENDRON [1]

B. H. DAVIS [2]

(WITH 2 FIGURES)

During the last three years frequent collections of *Rhododendron* leaves from several New Jersey plantings have been examined for leaf-spotting fungi. Examinations early in the work revealed the common occurrence of two pycnidial forms both of which possess characteristics in common with those of the genus *Phyllosticta*. One of these has large, granular, ovoid to globose-elliptical spores, whereas the spores of the other are small and dumbbell-shaped. It became apparent very soon that these were often associated in the same or in similar lesions. Later a perithecial stage was found intermingled with both *Phyllosticta* forms. The association of these forms suggested that they were the pycnidial, spermagonial, and perithecial stages of the same fungus. Proof of this and the identity of these three stages are reported in this paper.

LEAF SYMPTOMS, HOSTS, AND CULTURAL CHARACTERISTICS

The lesions in which any one or all of the above-mentioned types of fruit bodies occur are usually marginal but may occur anywhere on the leaf blade. Marginal lesions range from 3 mm. to 4 cm. in length and from 2 mm. to 2 cm. in width. Often the coalescing of spots forms a continuous necrotic lesion along most of the margin.

[1] Journal Series paper of the N. J. Agricultural Experiment Station, Rutgers University, Department of Plant Pathology.

[2] The writer is indebted to Dr. Anna E. Jenkins, Mycology and Disease Survey, U. S. Department of Agriculture, for help in connection with examination of specimens and checking of literature, and to Dr. C. L. Durham, Cornell University, for help in connection with the Latin names and endings. He is also indebted to Dr. John A. Stevenson, Mycology and Disease Survey, U. S. Department of Agriculture, and to Dr. F. J. Seaver, of the New York Botanical Garden, for their kindness in making specimens available for study.

Lesions in the center of the leaf are circular and range from 2 mm. to 2 cm. in diameter. The spots are reddish brown in contrast to the light brown to tan color of lesions resulting from sunburning or winter injury. The margins of the spots are only slightly darker than the central portions (FIG. 1, *A*).

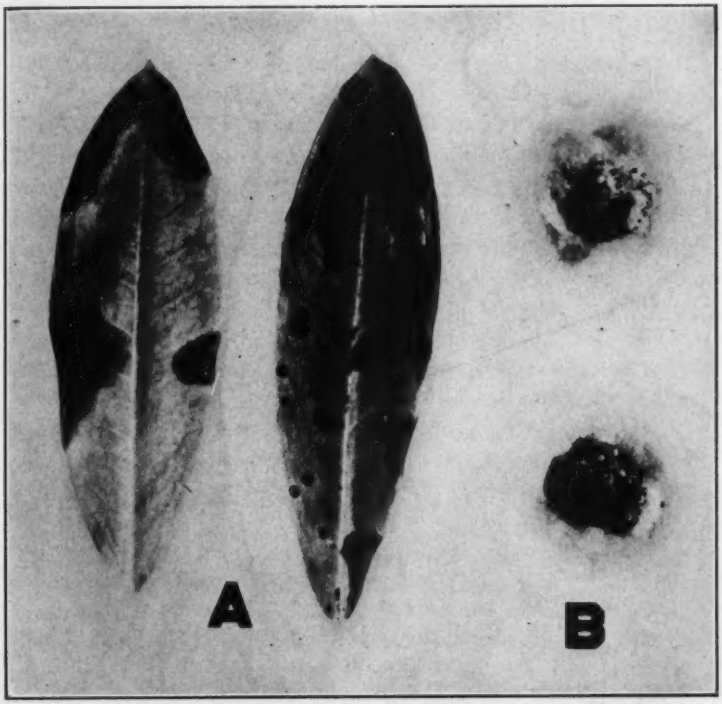

FIG. 1. *Guignardia Rhodorae*. *A*, leaf lesions containing perithecia, pycnidia and spermagonia, ⅔ natural size. *B*, cultures derived from ascospores on potato-dextrose agar, 2 weeks old, natural size.

One or more of the various stages of the fungus have been found on *Rhododendron maximum* L., *R. carolinianum* Rehd., *R. catawbiense* Michx., and some of the *catawbiense* hybrids. Weiss (13) records the fungus on *R. nudiflorum* Torr.

Cultures of the fungus have been derived from tissue plantings, pycnospores, and ascospores. The individual hyphae appear hya-

line, but the colonies, when cultured on potato-dextrose agar, are slightly yellowish. The fungus is slow-growing. After a period of 2 to 3 weeks colonies which usually contain mature pycnidia are 2 to 3 cm. in diameter (FIG. 1, *B*). Spermagonia and perithecia have not been found in agar cultures.

PYCNIDIAL STAGE

The pycnidial stage of the fungus appears as epiphyllous (rarely hypophyllous), black, spherical, slightly prominent bodies. Although they may be found sparsely scattered over the entire lesion, they are sometimes more numerous near the margins. They range in size from 125 to 200 μ in diameter, usually 140 to 150 μ. The spores, which are produced on slender stalks, are ovoid to globose-elliptical (sometimes pyriform), hyaline, granular, and range in size from 11.5 to 17.5 × 7.5 to 9.5 μ, usually 13.0–14.5 × 8.5 μ (fresh material).

The pycnidial stage has characteristics in common with *Phyllosticta maxima* described by Ellis and Everhart (**3**) in 1888. Their description follows:

Phyllosticta maxima Ellis & Ev.—on leaves of *Rhododendron maximum,* Bedford, Mass., July '83. Coll. Rev. Thos. Morong, Com. A. Commons.

Spots large, reddish-brown with a darker margin, mostly terminal or lateral, (3–5 cm.). Perithecia scattered, epiphyllous minute (½ mm.), their sub-acute apices slightly prominent. Sporules globose-ellipitical, hyaline, granular, 10–12 × 6–8 μ on rather slender pedicels about equal in length to the diameter of the sporule. The fruit is much like that of *P. sphaeropsoidea* Ellis & Ev. and the habit that of *P. terminalis* Ellis & Martin.

In 1892 Ellis and Everhart (**4**) reported *P. Rhododendri* West. on *Rhododendron catawbiense* based on a Newfield, New Jersey, collection of April 20, 1891. The spores are described as narrowly and acutely elliptical and 15–20 × 6–7 μ. Later in 1900 in their publication, North American Phyllostictas (**5**), they describe the spores as narrow-elliptical and 10–20 × 6–7 μ. Number *2765* of North American Fungi, collected at Newfield, New Jersey, in April 1891 is given as a specimen and the name *P. maxima* recorded as a synonym.

In the original description of *P. Rhododendri* as given by Saccardo (**9**), Westendrop describes the spores as cylindrical-ovate (no measurements given). The narrow-elliptical spores (15–20 × 6–7 μ) in Ellis and Everhart's description of *P. Rhododendri* and specimen No. *2765* of North American Flora (FIG. 2, *E*) compare favorably with the original description. However the granular globose-elliptical spores, 10–12 × 6–8 μ, in the original description for *P. maxima,* which they later record as a synonym, do not agree with either their or Westendrop's description of *P. Rhododendri.*

The writer has been unable to locate an Ellis and Everhart specimen which bears data corresponding to those given with the original description of *P. maxima.* Dr. W. L. White reports that the Herbarium of Cryptogamic Botany at Harvard does not contain the type specimen, and the writer does not find a packet bearing the exact data in the Herbarium of the New York Botanical Garden. However there exists in the latter a specimen labelled *P. maxima* which was collected by Thos. Morong and sent to Ellis and Everhart by A. Commons. The packet is marked "Ex-Herb. A. Commons No. *851.*" This specimen was collected at Medford, Massachusetts, and bears the date July–Aug. 1879, whereas the type material was collected at Bedford, Massachusetts, in July 1883. Although this cannot be considered the type, it may be considered an authentic specimen determined by Ellis. Spores from this collection are granular, globose-elliptical (FIG. 2, *A*) and 10–14 × 7–8.7 μ, which is slightly larger than that given by Ellis and Everhart (10–12 × 6–8 μ). Only a few spores were found. Our New Jersey specimens have larger spores, 11.5–17.5 × 7.5–9.5 μ, when collected. However, the size of spores in dried specimens is slightly smaller. Thus the writer's material agrees well with the Commons collection in the size, shape, and granular nature of the spore.

The Herbarium of the New York Botanical Garden contains two other specimens from the Ellis Herbarium both of which are labelled *P. Rhododendri* West. One of these is specifically the same as the writer's material whereas the other contains elliptical spores which are longer and narrower, 15–20 × 6–7 μ. The latter is specifically the same as specimen No. *2765* of North American

Flora (FIG. 2, *E*) labelled *P. Rhododendri* in the herbaria of the New York Botanical Garden, the New Jersey College of Agriculture, and the mycological collections of the Division of Mycology and Disease Survey. Another Ellis and Everhart specimen de-

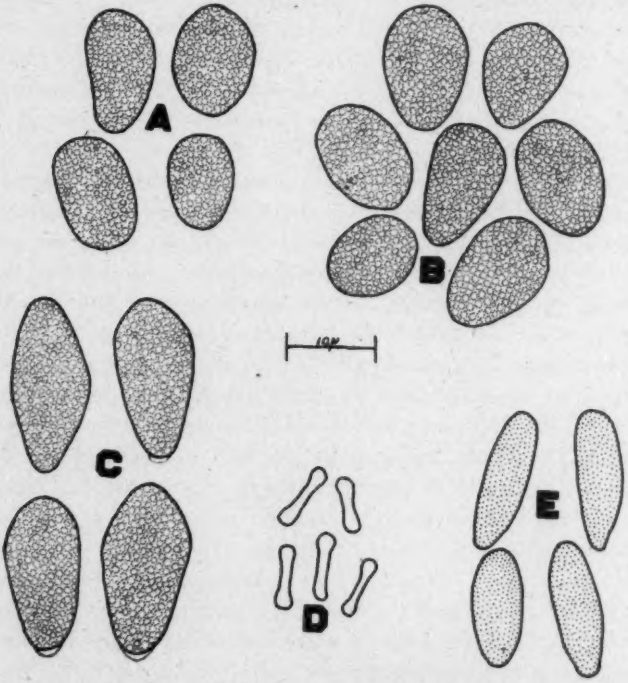

FIG. 2. *A, Phyllosticta maxima.* Spores from Ellis and Everhart's specimen. *B–D,* pycnospores, ascospores, and spermatia of *Guignardia Rhodorae* from New Jersey collections. *E,* spores from North American Fungi No. *2765, P. Rhododendri.* Drawn with aid of camera lucida.

posited in the last named herbarium labelled *P. Rhododendri* is specifically the same as No. *2765* of North American Flora.

Thus two Ellis specimens which are specifically the same, one labelled *P. maxima* and the other *P. Rhododendri,* have granular, globose-elliptical spores. The characteristics of this fungus agree with the original description of *P. maxima.* This is especially true since the original description states that the "fruit is much

like that of *P. sphaeropsoidea* Ellis & Ev. . . ." In addition to No. *2765* of North American Fungi, two other specimens from the Ellis Herbarium labelled *P. Rhododendri* have narrow-elliptical, non-granular spores. These agree with Ellis and Everhart's description of this fungus.

The writer considers *P. Rhododendri,* as represented by No. *2765* of North American Fungi (FIG. 2, *E*), and *P. maxima* (FIG. 2, *A*) as distinct species and feels that Ellis and Everhart were not justified in reducing their name, *P. maxima,* to a synonym of *P. Rhododendri* West. It is to be noted that Seaver (**11**) also questions this synonomy.

Although the original spelling of the specific name of Ellis and Everhart's species is *"maxima,"* Saccardo (**10**) changed it to *"Maximi."* It seems probable that Ellis and Everhart had in mind naming their *Phyllosticta* for the specific name of its host, *Rhododendron maximum.* If this is true Saccardo is correct in that *"Maximi"* means a *Phyllosticta* "of the giant" with the noun, *Rhododendron,* implied. Such a change is permissible under the International Code of Nomenclature. However, it is possible that Ellis and Everhart had in mind "a giant *Phyllosticta*" which would thus call for the spelling *"maxima."* Although the former interpretation is more likely, the latter is possible and the writer feels, therefore, that it is preferable to retain the original spelling.

SPERMAGONIAL STAGE

The spermagonia usually develop later than the pycnidia. However, mature spermagonia may be found in lesions containing both young and old pycnidia as well as perithecia. They are small spherical black bodies ranging in size from 60 to 125 μ in diameter. The spermatia which are produced on slender stalks are long and narrow, 5.0–8.5 × 1–1.5 μ. Their knob-like swollen ends, which become less prominent after drying, give them the appearance of a dumbbell (FIG. 2, *D*). Under moist conditions they exude from the spermagonia and form a small amber-colored drop at the ostiolum. All attempts to germinate these spores have failed.

Several species of *Phyllosticta* with small spores have been described on *Rhododendron.* Of these the characteristics of *Phyllosticta Saccardoi* Thüm. compare favorably with those of the

spermagonia described above. The writer has examined No. *1786* of De Thuemen's Mycotheca Universalis labelled *P. Saccardoi* at the New York Botanical Garden and the mycological collections of the Division of Mycology and Disease Survey and has found that the fruit bodies are similar. Unfortunately no spermatia were found. Dr. R. P. White reports by letter that he and Dr. C. L. Shear found the dumbbell-shaped spores in the type material of *P. Saccardoi* and that they are identical with the spermatia in question. Specimens in Dr. White's collections identified after this date and identical with the writer's fungus are labelled *P. Saccardoi* Thüm. The spermagonial stage is considered to be specifically the same as *P. Saccardoi* Thüm.

The name *P. Rhododendri* Sacc. (not Westendrop) is given by Saccardo (Syll. Fung. **3**: page 23) as a synonym of *P. Saccardoi.* Several other small-spored phyllostictas have been reported on *Rhododendron* some of which may also be identical with *P. Saccardoi,* but the writer has not had an opportunity to check authentic material. One of these is *P. Rhodorae* (Cooke) Tassi, which Cooke (**2**) suggests in his original description may be the spermagonial stage of *Sphaerella* (*Laestadia*) *Rhodorae* Cooke. Since the latter is shown by the writer to be connected with *P. Saccardoi, P. Rhodorae* may be a synonym. This fungus has been reported from Oregon by Zeller (**15**).

PERITHECIAL STAGE

The association of a perithecial stage in the same lesions with the pycnidial and spermagonial stages and the occasional occurrence of spermatia and pycnospores in the same pycnidia strongly suggested that all three of these forms were merely stages of the same fungus. Pieces of leaves containing perithecia were placed in drops of water on the underside of a lid of a petri dish containing potato dextrose agar. After the ascospores were shot, pieces of agar containing several spores were transferred to other petri dishes with agar. The spores germinated in a few hours, producing germ tubes most of which died after growing 2 or 3 mm. directly down into the agar. However a few continued to grow and pure cultures were obtained.

Plantings of mycelium derived from ascospores were made on sterilized strips of *Rhododendron* leaves in test tubes with moist cotton in the bottom. Examinations of these after a period of a month showed the presence of perithecia and also the pycnidia and spermagonia described previously. Perithecia were not obtained in all cultures made. This may have been due either to the lack of production or to the improper timing of the examinations. The same type of culturing was repeated using cultures derived from pycnospores, with the same results. Furthermore it has been noted that one of Ellis and Everhart's specimens of *P. maxima* also contained immature perithecia with characteristics similar to those described above. Thus the pycnidia, spermagonia, and perithecia are merely stages of the same fungus.

The perithecia are epiphyllous, globose to depressed-globose and are embedded in the leaf tissue with only the ostiolum extending through the epidermis. Those which develop in moist chambers are partially erumpent. The wall of the perithecium, which is thicker near the ostiolum, is 11.5–19.5 μ in thickness and is composed of brown pseudoparenchymatous cells. Perithecia range from 125 to 200 μ in diameter. The asci are 80–120 $\times$ 12.5–16 μ, fasciculate, 8-spored, clavate, rounded at their tips and possess short stalks. The wall is thickened at the apex. The asci extend to almost twice their original length at the time the spores are shot. No paraphyses have been found. The spores are subbiseriate, granular, one-celled, broadly elliptical (sometimes subrhomboidal) in shape, and measure 15–19 $\times$ 7.3–10.2 μ (FIG. 2, *C*). Many of the ascospores possess a detectable basal and a very faint apical pseudo-cell.

The characteristics of the ascigerous stage are identical with those of the genus *Guignardia* which is considered by Miller and Thompson (**6**) to be restricted to forms with uniloculate stromata, with no beak, fasciculate asci, no paraphyses and one-celled ascospores. Specimens were sent to Miller and Thompson, who found the fungus typical of the genus *Guignardia*.

The specific characteristics agree closely with *Sphaerella* (*Laestadia*) *Rhodorae* described by Cooke (**1**) on *Rhododendron* in England. His description follows:

Sphaerella (*Laestadia*) *Rhodorae* Cooke

Epiphyllous, perithecia gregarious, seated upon large irregular ferruginous spots, immersed in the parenchyma, subglobose, black, piercing the cuticle with the punctiform ostiola. Asci clavate, without paraphyses (.80–.120 × .16). Sporidia elliptical, hyaline, continuous (perhaps .015 × .007 mm. but too immature to measure with certainty).

On living leaves of *Rhododendron*. Kew.

This can scarcely be the *Laestadia Rhododendri*, De-Not. of Saccardo's Sylloge.

It will be noted that the measurements of the immature spores reported in Cooke's description are the lower limits of those given for New Jersey specimens. However the size of immature spores from our collection more nearly approaches that given by Cooke. The perithecial stage is considered specifically the same as *Sphaerella* (*Laestadia*) *Rhodorae* Cooke, and the new combination is proposed.

Guignardia Rhodorae (Cooke) comb. nov.

Syn. *Sphaerella* (*Laestadia*) *Rhodorae* Cooke
Laestadia Rhodorae (Cooke) Berl. & Vogl.
Phyllosticta maxima Ellis & Ev.
Phyllosticta Saccardoi Thüm.
Phyllosticta Rhododendri Sacc. (not West.)

Our New Jersey specimens of *G. Rhodorae* are specifically the same as the following deposited in the Herbarium of the Division of Mycology and Disease Survey of the Bureau of Plant Industry:

Laestadia Rhodorae (Cooke) Berl. & Vogl.
Brooklyn Bot. Garden, N. Y.; July 25, 1916.
Col. G. M. Reed; Det. A. E. J. and F. W. P.
Laestadia Rhododendri De-Not.
Arlington Farms, Va.; May 29, 1915.
Col. J. T. Rogers; Det. G. R. Lyman.
Laestadia Rhododendri De-Not.
Germantown Nurseries, Pa.; June 4, 1892.
Col. Thos. Meeham; com. Dr. Rex.

Laestadia Rhododendri (De-Not.) Sacc. mentioned above and originally described as *Sphaerella Rhododendri* De-Not. has now

been placed in the genus *Physalospora* by Rehm (**8**). The spores are described as 35–48 × 8–12 μ.

The structures of *G. Rhodorae* on Rhododendron are similar to but in general larger than those of *G. Vaccinii* Shear described on *Vaccinium* and reported on *Kalmia latifolia* from New York by Welch (**14**). The spermagonia and pycnidia of *G. Rhodorae* also appear larger than corresponding structures of a similar fungus collected by Dr. R. P. White and the writer on *Kalmia* in New Jersey. No pycnidia of the *Kalmia* fungus have been found by the writer. Although this fungus may be identical with *G. Rhodorae,* the size of its structures appears to agree more nearly with those of *G. Vaccinii.*

It is to be noted that the pycnidia, spermagonia, and perithecia of *G. Rhodorae* are similar to but larger than those of *Guignardia Bidwellii* (Ellis) Viala & Ravaz. The ascospore of *G. Bidwellii* has a pseudo-cell at one end which is described by Reddick (**7**) as an inflated hyaline vesicle. He agrees with Prilleaux and quotes the latter in saying "that they bear at their extremity a little mucilaginous material, inflated and transparent, which probably aids in fixing them to the leaf where they may germinate." The same type of structure is visible on the basal end of most of the ascospores of the *Rhododendron* fungus, but much less prominent than in *G. Bidwellii.* A similar structure is sometimes faintly detectable at the apical end. Examinations of immature perithecia which have been crushed under a cover glass show that young ascospores forced from the ascus have cytoplasmic contents of the ascus adhering to them, suggesting that the mucilaginous material may be derived from adhering cytoplasmic contents.

In 1924, T. A. Tengwall (**12**) reported *Venturia Rhododendri* Tengwall as the perfect stage of *Phyllosticta maxima.* He states that isolations from leaves received at the Phytopathological Laboratory at Baarn yielded two fungi, *Pestalozzia guepini* and *Phyllosticta rhododendricola* Brun., which produced dark colored mycelium on cherry agar.. Inoculation experiments carried out with the latter gave positive results and a few of the infected leaves which fell from the inoculated plants were placed on filter paper in a petri dish. Later, these were sectioned and the presence of *P. rhododendricola* was demonstrated. The author states that two

other species were also found on these fallen leaves, *P. maxima*, which was sometimes found in the same fruit body with *P. rhododendricola*, and a pyrenomycete which he described as new under the name *Venturia Rhododendri*. On the basis of the above association, *V. Rhododendri* was considered the perfect stage of *P. maxima* and *P. rhododendricola*. Tengwall also considers the names *P. berolinensis* P. Henn. and *P. rhododendri-flavi* Bub. & Kab. as synonyms.

The perfect stage which Tengwall figures and describes is clearly a species of *Venturia* and distinctly different from that of the writer's. It is assumed that the conidial fungus which he determined as *P. maxima* and considers the imperfect stage of *V. Rhododendri*, is specifically different from that which the writer found to be identical with Ellis & Everhart's specimens of *P. maxima*.

SUMMARY

In this study an ascigerous stage, originally described as *Sphaerella* (*Laestadia*) *Rhodorae* Cooke, is connected by means of pure culture methods with its pycnidial stage *P. maxima* Ellis & Ev. and its spermagonial stage *P. Saccardoi* Thüm. The characteristics of the perithecial stage are considered to be those of the genus *Guignardia* and the name *G. Rhodorae* (Cooke) comb. nov. is proposed. Synonyms are listed.

P. maxima is considered a distinct species and not synonymous with *P. Rhododendri* West. as reported by Ellis and Everhart. It is also considered distinct from the fungus determined by Tengwall as *P. maxima* and reported by him as the imperfect stage of *Venturia Rhododendri* Teng.

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ADDITIONS TO THE FUNGI IMPERFECTI ON GRASSES IN THE UNITED STATES [1]

RODERICK SPRAGUE [2]

(WITH 2 FIGURES)

The fungi listed in this article have all been collected by the writer in the western United States except the first three species.

Septoria digitarivora sp. nov.

Maculis ellipticis, v. diffusis, brunneis, tarde centro subalbidis; pycnidiis conspersis, sparsis, brunneis, ostiolatis, erumpentibus, subglobosis v. subellipsoideis, 110–128 μ, parenchymaticis; pycnosporulis clavato-filiformibus, apicis acutis, basis subobtusis, 3–7 septatis, 45–95 × 3.6–5.6 μ.

Hab. in foliis vivis *Digitariae sanguinalis* (L.) Scop., West Virginia.

Spots elliptical, finally diffuse, brown or fuscous, later isabelline in the centers; pycnidia scattered, few to several to a lesion, seldom gregarious or two or three may be grouped, brown parenchymatous, erumpent, subglobose to subellipsoidal, 110–128 μ, ostiole small; pycnospores clavate-filiform, apex acuminate, base subobtuse, 3- to 7- often 5- to 7-septate, contents somewhat coarse, hyaline, but with yellow or chlorine inclusions, 45–95 × 3.6–5.6 μ, but mature spores are mostly 70–95 × 3.6–4.5 μ. Mean spore size 84 × 4 μ, ratio of length to width 21 to 1.

In living leaves of *Digitaria sanguinalis* (L.) Scop., A. D. Hopkins, Mineral Wells, W. Va. (Without date, 1943.)

The spores of this species (FIG. 1, *a*) are much like those of *Phaeoseptoria Urvilleana* (Speg.) Sprague (12) except that they

[1] Coöperative investigation between the Divisions of Cereal Crops and Diseases, Forage Crops and Diseases, Dry Land Agriculture, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration; Division of Nurseries, Soil Conservation Service, U. S. Department of Agriculture, and the North Dakota Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering.

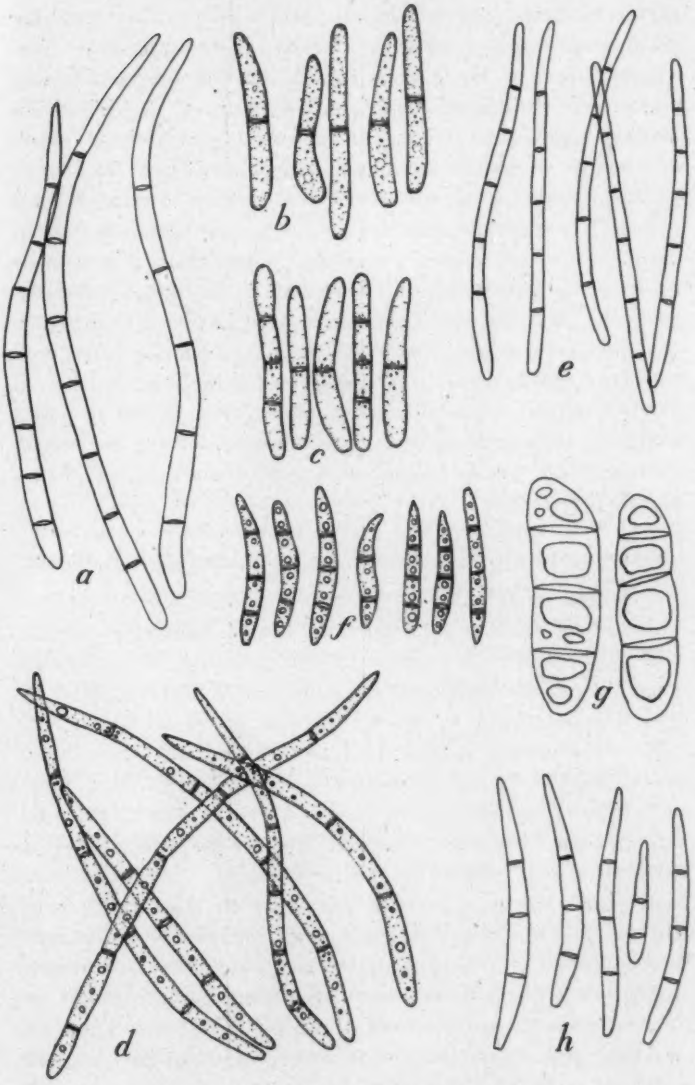

FIG. 1. Spores of *Septoria* and *Stagnospora*.

are somewhat narrower and lack the brown color. They also have the general shape of those of *Septoria Andropogonis* var. *Sorghastri* Greene & Sprague (**6, 14, 16**) but they are considerably coarser than those of this fungus on *Sorghastrum*. *S. digitarivora* does not appear to be the same as any species previously described. The mature spores are sometimes as much as 24 times as long as wide and, therefore, are not related to *Stagonospora arenaria* Sacc. (**13**). It is surprising that this fungus has not been found previously, but the leaf spots of the eastern United States grasses have not been thoroughly studied. Seymour (**8**) reports *S. graminum* Desm. on *Digitaria*, but *S. graminum* (**17**) is very different from our specimen and it is doubted if the citation in Seymour's text refers to a fungus that is at all like the one collected by A. D. Hopkins. The type, which bears the number B. P. I. *80,869*, now filed in the Mycological Collections of the Bureau of Plant Industry at Beltsville, Md., was forwarded through the courtesy of J. G. Leach and K. W. Kreitlow.

SEPTORIA ARECHAVALETAE AND S. TANDILENSIS ON PANICUM

A fungus referable to *S. Arechavaletae* Wint. was collected on *Panicum capillare* L. in Virginia by David Fairchild, Aug. 16, 1889. The scattered pycnidia contain quantities of exceedingly long, filiform, obscurely septate, hyaline spores 50–115 × 1.0–1.5 μ. The type description by Winter reported spores 60–80 × 1.5–2 μ (**21**). Comparable species are *S. laxa* Speg. (**10**) with filiform spores 50–60 × 1 μ and *S. tandilensis* Speg. (**9**) with spores 40–60 × 1–1.5 μ. The first species described was *S. Arechavaletae* but it is suspected that all three species may be the same. The Virginia material is referred to *S. Arechavaletae*. Unfortunately the type of this species is not available and a study of the pycnidial characters of the group indicates that *S. tandilensis* may also occur in the United States and may warrant recognition as a distinct species. *S. Arechavaletae* has sparse, subgregarious, totally immersed, globose pycnidia (100 μ) while *S. tandilensis* Speg. (**9**) was described as having somewhat more gregarious, black cupulate, collapsed pycnidia. Most of the eastern collections seen have the typical black pycnidia of *S. tandilensis* but with somewhat too long spores as based on Spegazzini's description. The United States

material has spores as much as 80–90 μ long. Material has been seen from Virginia (Diehl), Wisconsin (J. J. Davis and Greene) and along the coast to Florida on various species of *Panicum*. Most of these stray collections, long undetermined, from the Atlantic coastal region are devoid of pycnospores but the pycnidia contain prosenchymatous material. No maturing perithecia however have been found. Any of this material is readily identified by the grouped black pycnidia, which are somewhat cupulate-collapsed in the winter condition.

Until the types can be compared it is better to call the common fungus with the black pycnidia *S. tandilensis* and refer Fairchild's specimen to the earlier described *S. Arechavaletae*.

S. sigmoidea Ellis & Ev. (**4**) from Iowa is not a species of *Septoria* but is near *Hendersonia crastophila* Sacc. *Septoria Donacis* Pass. var. *Panici* Ellis & Barth., *nomen nudum*, has falcate spores and is a narrow-spored form of *Selenophoma Donacis* (Pass.) Sprague & A. G. Johnson. *Septoria Urvilleana* Speg. on *Panicum Urvilleanum* Kunth. from the Argentine is elsewhere placed in *Phaeoseptoria* (**12**). Seymour lists *S. graminum* J. J. Davis, non Desm. on *Panicum depauperatum* Muhl. (**8**). Davis (**3**) in 1942 listed *S. tandilensis* on this host, stating that is is perhaps not distinct from *S. graminum*. This material is what the writer agrees is *S. tandilensis*.

SEPTORIA MELICAE PASS.

S. Melicae was found on *Melica scabrosa* Trin. in field plots at Mandan, N. Dak. It produced white elliptical spots with brown borders on above-ground parts of maturing plants. The pycnidia were yellow-brown, subglobose, 80–120 μ diameter and contained bacillar spores 22–38 × 2.8–3.8 μ (FIG. 1, *b*). Some of the spores were mature and 3-septate but most of them were 1-septate partly developed summer spores. This fungus is very similar to a number of other fungi including *Septoria Avenae* Frank and *Stagonospora Arrhenatheri* A. L. Sm. & Ramsb. The latter is, we now believe, a synonym of *S. Avenae* as *S. Melicae* also may well prove to be.

Our specimen appears to be the first collection of this fungus on *Melica* in North America although Seymour (**8**) lists *S. Avenae*

on *Schizachne purpurascens* (Torr.) Swallen, which is a species of grass that was for many years listed under *Melica*.

SEPTORIA SECALIS VAR. STIPAE SPRAGUE

The type of this variety has 3-septate spores, 37–54 × 2.6–3.1 μ (**15**), but much of the summer material in North Dakota has 1- to 3-septate spores, 22–40 × 2.6–3.7 μ. These are from 8 to 10 times as long as broad, and represent a *Stagonospora*-like phase of the fungus. Morphologically they appear to be the same fungus as *S. Secalis* var. *Stipae* and are considered only a warm-season phase of this variety. Pycnospores from a collection on *Stipa Williamsii* Scribn. from the plots at Mandan, N. Dak. (B. P. I. *80,870*), are illustrated (FIG. 1, *c*). In this phase it is similar to the *S. Avenae* complex. Microspores, 5–10 × 0.5–0.7 μ, were found.

In addition, the writer has found a species of *Septoria* on *Stipa comata* Trin. & Rupr. at Mandan, N. Dak., which he refers to *S. Andropogonis* f. *sporobolicola* Sprague. The obclavulate spores are 40–73 × 2.4–3.4 μ (FIG. 1, *d*). This fungus was very common at Mandan in 1944. It produces a dark brown blotch, which is confusable with *Pseudomonas coronafaciens* var. *atropurpureum* (Reddy & Godkin) Stapp. It probably is more common than available collections indicate. Material on *S. comata* was obtained east of Crane, Mont., June 20, 1945 (B. P. I. *81,121*).

SEPTORIA SPARTINAE (TREL.) SPRAGUE

Saprophytic material similar to *S. Spartinae* occurs on dead culms of *Beckmannia syzigachne* (Steud.) Fern. in North Dakota at Buffalo Lake and near Carrington, usually associated with other fungi. In the Buffalo Lake material the pycnidia were small, 60–90 μ, black, deep-seated, somewhat longer than broad and contained hyaline (or faintly yellow in mass) 1- to 2-septate spores 35–44 × 2.9–3.3 μ. The spores are blunt at the base and taper to a sharp apex, and they are stiffly curved. They are slightly less cylindrical than those of *S. Spartinae* but are otherwise sufficiently close to permit assignment to that species. *Beckmannia* and *Spartina* have similar marshy habitats and both belong to tribe Chlorideae of the Gramineae. The only other *Septoria* found on related grasses is *Septoria Cynodontis* Fuckel, which has filiform

spores 50–65 × 1.5–2 μ and may therefore be eliminated from consideration.

SEPTORIA QUINQUESEPTATA SPRAGUE

This species, recently described (**14**), is herein illustrated (FIG. 1, *e*). Another collection on *Sphenopholis obtusata* from Devil's Lake, N. Dak., has spores close to *S. Andropogonis* J. J. Davis (FIG. 1, *h*) and is assigned to that species. *S. quinqueseptata* is related to the *S. Andropogonis* complex, but the material in the type appears to indicate that it has definitely narrower spores.

STAGONOSPORA FOLIICOLA (BRES.) BUB.

Syn.: *Stagonospora vexata* Sacc. var. *foliicola* Bres.
St. vexata var. *Baldingerae* Sacc.

We have seen several collections of a *Stagonospora* on *Phalaris arundinacea* L. from the United States (Iowa, Ky., Minn., N. Dak., Wis.) with spores 27–45 × 2.7–4.1 μ. These were referred with some hesitation to *Stagonospora arenaria* Sacc. (**13**). Recently the writer finally obtained mature spores of the *Stagonospora* on *Phalaris* from abundant material in the plots at Mandan, N. Dak. The spores were 38–67 × 4.6–6 μ, stoutly obclavulate, with a tapering, finally blunt base and an acute apex, 3- to 8-septate, sometimes, but less often not, constricted at the septa (FIG. 2, *a*). This fungus is *Stagonospora foliicola* (Bres.) Bub. (**1**), which is common in Europe. It produces an elliptical, brown to vinaceous spot, which later becomes diffused as a wine colored blotch on the leaves. John A. Stevenson sent material of J. Smarod's Fungi Latvici Exsiccati No. *441*, which had spores 47–56 × 4.5–5.8 μ and they were mostly 7-septate. He also sent *St. vexata* var. *Baldingerae* from Vestergren's Micromycetes Rariores Selecti No. *1443* from Mércy-sur-Seine, France, which fungus is only a saprophytic phase of *St. foliicola*.

St. foliicola is readily obtained in pure culture on potato dextrose agar. It forms a slow-growing, white, cottony growth with pink color in the substratum. It is much slower growing than *St. arenaria*. It has, to date, remained sterile in pure culture on *Melilotus* culms.

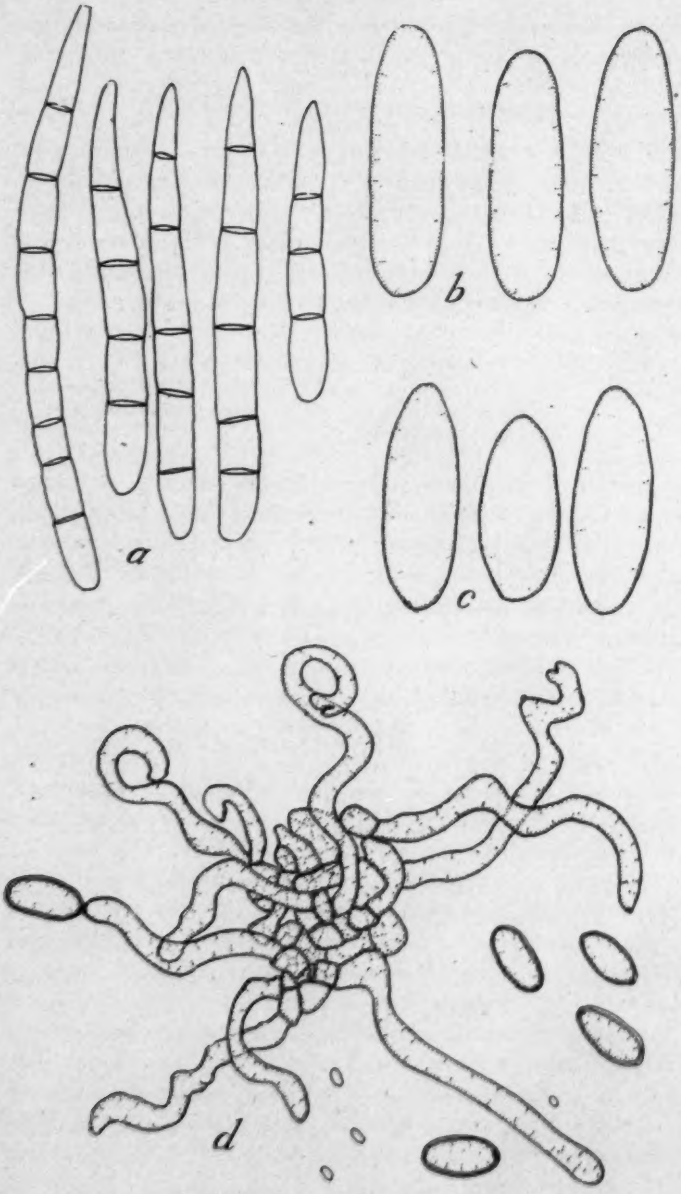

FIG. 2. Spores of *Stagnospora*, *Macrophoma* and *Ovularia*.

Abundant material of *St. foliicola* from Mandan has been sent to the Division of Mycology and Disease Survey, Bureau of Plant Industry, Beltsville, Md., for distribution. *St. foliicola* also occurs on *Phalaris californica* Hook. & Arn. in Marin County, Calif. (W. B. Cooke).

There appear to be some differences in the susceptibility of clones and of strains of *Phalaris arundinacea* to this fungus in the plots at Mandan. The plots are on sandy upland ground. The fungus usually does not appear in abundance until August but by early September it occurs on almost 100 per cent of the leaves of susceptible plants.

STAGONOSPORA SIMPLICIOR SACC. & BERL.

Saprophytic material was abundant on *Calamovilfa longifolia* (Hook.) Scribn. in mid-September, 1943, at Mandan, N. Dak., following light frosts (B. P. I. *80,884*). The symptoms were vague, stramineous or gray areas, and the pycnidia were scattered to grouped, black, globose and mostly 110–140 μ in diameter. The blunt pycnophores bore hyaline to faintly yellow, cylindrical or slightly bent, obtuse, 3-septate spores, which were slightly to scarcely constricted at the septa (FIG. 1, *g*). The spore contents were coarse, sometimes consisting mainly of one huge cell inclusion. The spores were 28–35 × 8–9 μ as compared with 32–38 × 8–9 μ for the type on *Phragmites communis* in France.

St. simplicior f. *Andropogonis* Sacc. was described from North Dakota material (Brenckle). H. C. Greene sent material to us of a fungus on *Sorghastrum nutans* (L.) Nash from Madison, Wis., which he determined as this variety (**6**). It appears to be parasitic in elliptical spots on living leaves. *St. simplicior* is likely to prove rather widespread on coarse grasses in the prairie regions, but except for the Wisconsin material has not been shown to be associated with living host parts.

STAGONOSPORA AGROSTIDIS FORMA ANGUSTA f. nov.

Maculis fuscis v. stramineis v. nullis, pycnidiis globosis, aureis, parenchymaticis, ostiolatis, 120–280 μ; pycnosporulis fusiformibus, subacutis, rectis v. curvulis, basis subobtusis v. subtruncatis, non constrictis, 3-septatis, hyalinis, conspicue guttulatis (4–8), 15–27 × 2.2–3.2 μ (medio 22.3 × 3 μ).

Hab. in foliis et vaginis *Stipae viridulae*, 22 miles n. of Beach, N. Dak., July 10, 1940 (B. P. I. *80,976*).

We also have material on *Elymus canadensis* L. collected at Foster, Warren Co., Ohio, by Wm. Bridge Cooke (*14,538*), which has spores virtually identical with the fusoid, cylindrical to rarely subfalcate ones of this species. This material, however, is associated with *Phyllachora graminis* (Pers.) Fuckel and is distinct from the *Stipa* material. Other collections than the type from North Dakota are associated with rust pustules or in some cases with summer material of *Septoria Secalis* var. *Stipae*. It is distinguished from the 1-septate phase of the latter (FIG. 1, *c*) by the somewhat curved, fusiform or fusoid spores (FIG. 1, *f*) which are nearly all smaller than the smallest of the bacillar spores of *S. Secalis* var. *Stipae*. In old material the spores of the *Stagonospora* take on a yellow tinge. They are, however, far lighter in color than their nearest affinity in *Hendersonia, H. simplex* Schroet.

There are a number of other fungi described that show similarity in some characters with *St. Agrostidis* f. *angusta* but which differ in others. *St. insularis* Speg. on *Agrostis magellanica* has cylindrical to subclavate spores 18–24 × 3 μ, but the pycnidia are small, 80–90 μ, lenticular and fuliginous, too different to indicate relationship. *St. Agrostidis* Syd. (18) on *Agrostis vulgaris* is closer to *St. Agrostidis* f. *angusta* as the pycnidia are 180–250 μ in diameter, and the 3-septate spores are fusoid, curved to subfalcate with acute ends. The spores, 24 × 4 μ, appear somewhat wider than our form and for that reason we are segregating the *Stipa* material to the extent of naming it as a form of Sydow's species.

It should be mentioned that *St. smolandica* Eliasson on *Agrostis vulgaris* is very similar to *St. insularis*. It is associated with sterile stroma of *Phyllachora graminis*. The spores are cylindrical, and the species is evidently not at all related to *St. Agrostidis* f. *angusta*. The latter is also distinct in several ways from *Septoria nodorum* Berk. *S. nodorum* has cylindrical to subfusoid spores, typically rounded at both ends and usually slightly to distinctly constricted at the septa. The contents are not coarsely guttulate and in addition the pycnidia of *S. nodorum* are much smaller than most of those of the *Stagonospora*.

The *Stipa* material belongs in *Stagonospora* because the spores are definitely broadened, less than eight times as long as wide.

MACROPHOMA PHLEI TEHON AND STOUT

A fungus which we call *M. Phlei* was found on dying leaves of *Elymus triticoides* Buckl. in the plots at Mandan, N. Dak. (B. P. I. *80,893*). The fungus is of no economic importance and the material is scanty. It occurs in diffuse straw-colored spots. The pycnidia are black, depressed-globose, tardily ostiolate (12–16 μ), arranged in lines along the veins of the leaf, 120–135 $\times$ 120–204 μ, and the pycnospores are elliptical to elongate ovate, slightly larger at one end, hyaline with pure white, opaque contents, 28–39 $\times$ 8.5–11 μ, mean size 32 $\times$ 9.4 μ.

This fungus resembles *M. Phlei* in a number of ways (**20**). The pycnidia, their arrangement, the shape if not the size of the spores are all very similar in the Illinois and North Dakota material. Our collection is distinguished somewhat from the type by its larger spores (FIG. 2, *b*). Those of the type are 18–26 $\times$ 6.4–7.7 μ and since Tehon and Stout report that their fungus is evidently mature, the material from Mandan, which also appears to be mature, may have spores too large for *M. Phlei*. The smallest spore in our material is somewhat larger than the largest of *M. Phlei*. The writer has seen similar material on *Agrostis palustris* from Newport, Oregon, with spores 24–31 $\times$ 9.5–11 μ (FIG. 2, *c*). Because all material seen is scanty, we prefer to leave our collections in *M. Phlei* but the description of the latter should be modified to conform to the range of the spore size indicated.

M. Zeae Tehon & Daniels has fusiform, hyaline to greenish spores 17–31 $\times$ 6.5–8.5 μ borne in extensive spots with the pycnidia (65–120 μ) scattered throughout the spots. *M. Zeae* (**19**) therefore appears to be very different from *M. Phlei*.

CERCOSPORA BROMI SPRAGUE

Cercospora Bromi Sprague (**11**) is notable for the peculiar secondary conidia, which are attached and down-deflected from the first or second basal cell of the conidia. On the basis of Newhall's study (**7**) of a fungus that causes a disease of celery, our fungus

should be placed in the genus *Ansatospora* Newhall. Therefore **Ansatospora Bromi** (Sprague) comb. nov. is proposed for the fungus on *Bromus rigidus* Roth.

OPHIOCLADIUM HORDEI CAV.

On the basis of material from Wisconsin, which Davis (3) and later Greene (5) assigned to *Ophiocladium Hordei* Cav., the writer refers comparable material on the same host, *Phalaris arundinacea* L., from Moskee, Wyo., and Spearfish Creek, S. Dak., to this species. This fungus appears to be relatively common in favored locations along creeks. It produces amphigenous straw-colored to buff, later pale-brown spots on the leaves which later become chalky white with the fruiting material of the fungus. The spots when mature are elongate, striate, often 1–2 × 10–30 mm. and finally are more or less confluent and covering or killing most of the leaf surface. They are emarginate or with diffuse yellow-buff surrounding the lesions. The conidiophores are in compact fascicles in neat rows between the veins of the leaf, emerging from stomata and arising from yellow to mostly hyaline compacted or serpentine hyphae. These strikingly distinct conidiophores (FIG. 2, *d*), which Cavara (2) certainly does not illustrate as being serpentine, are 3–4.5 μ in diameter, 20–40 μ tall, tortuous, twisted, or remarkably serpentine, particularly at the apex. The conidia are produced at the apex or on the sides of conidiophores adjacent to the apex. The spores show a slight hilum after breaking away from the conidiophores. The spores are hyaline, 1-celled with an evident wall (as shown by Cavara), which appears faintly roughened, 11–15.5 × 6–7 μ. The spores are not numerous. Associated are smaller hyaline spores.

This fungus differs from *O. pulchella* (Ces.) Sacc. in the unusually serpentine conidiophores, the elongate-striate, not oval, lesions and in the structure of the spore wall.

O. Hordei is known as an obscure disease of barley in northern Europe. It has not been reported on barley in the United States and whether critical comparison with European material on barley will disclose anything more than racial differences must await possible later study.

Ophiocladium is generally placed under the *Micronemae* with their "hyphae very short or obsolete, or little different from the conidia." This is obviously incorrect. Under *Macronemae* this species keys to *Ovularia*. The differences between this fungus and well recognized species of *Ovularia* are insufficient to warrant recognition of the fungus in *Ophiocladium*. The writer sees no need for the genus *Ophiocladium*, which he proposes to reduce to synonymy under *Ovularia*. He therefore proposes **Ovularia Hordei** (Cav.) comb. nov.

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EXPLANATION OF FIGURES

FIG. 1. (All × 1000.)

a. Pycnospores of *Septoria digitariivora* Sprague, from type.
b. Pycnospores of *Septoria Melicae* Pass. on *Melica scabrosa* Trin., Mandan, N. Dak.
c. Pycnospores of *Septoria Secalis* var. *Stipae* Sprague on *Stipa Williamsii* Scribn., Mandan, N. Dak. This represents the 1- to 3-septate summer phase of this species.
d. Pycnospores of *Septoria Andropogonis* f. *sporobolicola* Sprague on *Stipa comata* Trin. & Rupr., Mandan, N. Dak.
e. Pycnospores of *Septoria quinqueseptata* Sprague, type.
f. Pycnospores of *Stagonospora Agrostidis* f. *angusta* Sprague, from type.
g. Pycnospores of *Stagonospora simplicior* Sacc. & Ber. on *Calamovilfa longifolia* (Hook.) Scribn., Mandan, N. Dak. (B. P. I. *80,884*).
h. Pycnospores of *Septoria Andropogonis* J. J. Davis on *Sphenopholis obtusata* (Michx.) Scribn., Devil's Lake at Ft. Totten (station), N. Dak., Aug. 6, 1943.

FIG. 2. (All × 1000.)

a. Pycnospores of *Stagonospora foliicola* (Bres.) Bubák from *Phalaris arundinacea* L., Mandan, N. Dak. (B. P. I. *80,895*).
b. Pycnospores of *Macrophoma Phlei* Tehon & Stout from *Elymus triticoides* Buckl., Mandan, N. Dak.
c. Pycnospores of *Macrophoma Phlei* from *Agrostis palustris* Huds., six miles s. of Newport, Oregon, Dec. 18, 1937 (O. S. C. 39, W. B. Cooke coll.).
d. Fascicle of conidiophores and detached conidia of *Ovularia Hordei* (Cav.) Sprague on *Phalaris arundinacea* L., Moskee, Wyo.

ELSINOË ON RANDIA

ANNA E. JENKINS AND A. A. BITANCOURT

(WITH 1 FIGURE)

In 1937 in conversation with the senior writer, J. A. Stevenson recalled that a disease of similar symptoms to those of sour orange scab caused by *Elsinoë Fawcetti* Bitanc. and Jenkins, which he had studied in Puerto Rico, was often present on *Randia mitis* L., a common shrubby plant on the island. Upon request, W. A. McCubbin, then stationed at San Juan, made several collections of fruits of *R. mitis* showing abundant scabbing such as that Mr. Stevenson had remembered. On specimens consisting of branches with the fruit still attached it was noted that leaves and stems also were infected, but much less conspicuously and abundantly so than fruits. Cultures made by the senior writer from fruits sent in 1938 yielded typical vegetative growth of *Elsinoë*, and ascomata of this genus were discovered on the gathering of 1939, which was studied by the junior writer.

Evidence that "scab of Randia" is widespread and of long standing in the West Indies is afforded by the fact that typical lesions are present on various specimens of *Randia mitis*, as well as on other species including *R. parvifolia* Lam., in the U. S. National Herbarium, Washington, D. C., and the herbarium of the New York Botanical Garden, N. Y. Two of these specimens, one bearing the date 1877, will be cited in connection with the description of the *Elsinoë* on *Randia*, which follows:

Elsinoë puertoricensis Jenkins & Bitanc. sp. nov. FIG. 1.

Leaf spots generally few, circular to subcircular, measuring up to 1 mm. in diam., visible on both leaf surfaces, with the central part thin, parchment-like, translucent, often falling away, margin of well developed spots forming a salient ring, waxy in appearance, upper surface of spots including the nerves which traverse them "light vinaceous cinnamon," [1] "vinaceous cinnamon," or "cinnamon

[1] Names of colors in quotation marks are according to "color standards and color nomenclature" by Robert Ridgway (1912).

buff," becoming somewhat paler toward the margin, the surrounding leaf tissue water-soaked in appearance becoming "olivaceous black" and "fuscous black," fruit spots circular or nearly so, about 1 mm. in diam., scattered or confluent forming irregular areas, dirty white at the center, sometimes dotted by the dark ascomata of the *Elsinoë*, separated from the surrounding tissue by a fine, pale to dark line of demarcation, conspicuously raised and wartlike and surrounded by a more or less pronounced fissure or flattened with the border, of varying width, merging with that of adjoining lesions where spots are grouped; ascomata erumpent, measuring 50–150 μ in diam. by 20 to 50 μ in thickness, forming a pulvinate relatively homogeneous mass of pseudoparenchyma, often darkened in the peripheral region, merging into a hyaline prosenchyma which is intermingled with the superficial cells of the cicatricial periderm forming the external part of the lesion, without a well defined epithecium, asci irregularly distributed throughout the pseudoparenchyma including the peripheral region, in the specimens examined, occasionally protruding above the surface of the ascoma, globose with internal wall noticeably thickened in the apical region, faveola sometimes distinguishable, 14–20 μ in diam., containing 8 ascospores; ascospores hyaline, 1 to 3 septate, measuring 11–14 $\times$ 4–5 μ.

Maculae in foliis amphigenae, sparsae, circulares vel subcirculares, usque 1 mm. in diam., centro tenui translucenti, margine elevato, annuliformi, brunneo, ceraceo, interdum zona nigrescenti circumdatae; lesiones in fructibus dispersae vel in caespites irregulares aggregatae, subelevatae vel verruciformes, centro glabro albido, margine angusto pallido usque fusco interdum fissurato; ascomata erumpentia, 50–150 $\times$ 20–50 μ, pseudoparenchyma comparative homogeneum obscurum componentia, superficiem versus leniter obscuriora, epithecio non evoluto; asci in pseudoparenchyma parte peripherical inclusa irregulariter distributa, interdum ad superficiem ascomatis protrudentes, globosi usque obovati vel ellipticales, 14–20 μ in diam.; ascosporae usque 8, hyalinae, 3-septatae, 11–14 $\times$ 4–5 μ.[2]

[2] Latin diagnosis prepared by Miss Edith K. Cash.

FIG. 1. *Elsinoë puertoricensis* Jenkins & Bitanc. on *Randia mitis* from Puerto Rico. *A*, On leaves, fruits and stems of specimens from Arecibo, P. R., Mar. 7, 1939, W. A. McCubbin. *a*, leaf spot intact; *b*, leaf spot with center fallen away. $\times$ 1. *B–C*, Fruits from Vega Baja, Feb. 8, 1938, W. A. McCubbin. $\times$ 6. *D*, *F*, and *G*, Sections of ascomata from same specimen. *a*, asci with ascospores; *b*, malformed host tissues. $\times$ 500. *E*, Culture on potato medium (Thaxter). $\times$ 1. Photographs by M. L. F. Foubert (*A–C*, and *E*) and by Bitancourt (*D*, *F*, and *G*).

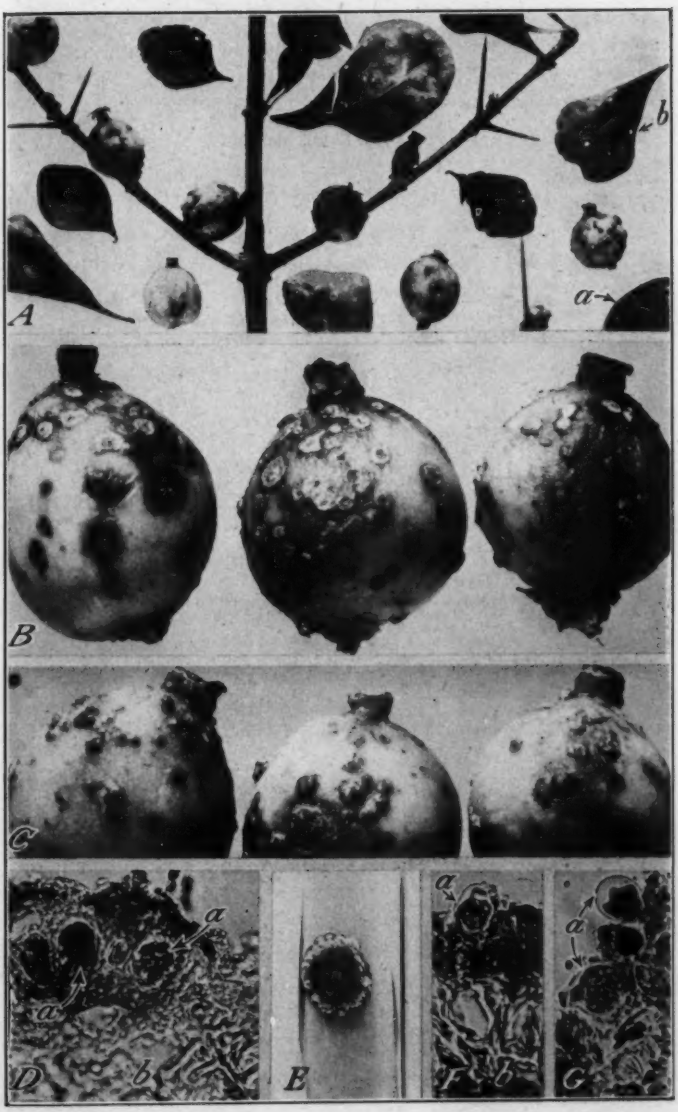

FIG. 1.

DISTRIBUTION: Producing a conspicuous scab on fruits of *Randia mitis* L. and other species of this genus of the Rubiaceae, including *R. parvifolia* Lam., leaves and stems also affected, West Indies including Martinique, Puerto Rico and Haiti.

SPECIMENS EXAMINED:[3] On *Randia mitis* L. Martinique: 1877, Père Duss 990 (USNH *845947*, fragment in USM); Puerto Rico: Palo Seco, Oct. 19, 1937, W. A. McCubbin (USM *72729;* IB *2965*); Vega Baja, Feb. 8, 1938, W. A. McCubbin. Type (USM *73729;* IB *2966*); Arecibo, Mar. 7, 1939, W. A. McCubbin (USM *73056;* IB *4391*); on *R. parvifolia* Lam., Haiti, Vic. Port de Paix, Dec. 22, 1928, E. C. Leonard 1103 (USNH *1450080*).

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[3] Symbols employed in citing specimens are as follows:

USM Mycological Collections of the Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md.

IB Secção de Fitopatologio do Instituto Biologico de São Paulo, São Paulo, Brazil.

USNH United States National Herbarium, Smithsonian Institution, Washington, D. C.

ISOLATES INTERMEDIATE BETWEEN STACHYBOTRYS AND MEMNONIELLA

ROBERT K. ZUCK

(WITH 2 FIGURES)

INTRODUCTION

Interest in this country in *Memnoniella echinata* (Rivolta) L. D. Galloway arose with reports from Australia that this fungus was being used for testing variously treated cotton cloth for mildew resistance. Subsequently, several workers have found that it is not rare in this country. The writer and his associates have frequently observed *M. echinata* on mildewed cloth removed from soil contacts as well as from above-ground exposures ranging from Madison, Wis., to New Orleans, La. That it is a rather common soil organism here is suggested by the fact that it occurs regularly on fabric test specimens removed from soil burial tests using Chester Loam from Maryland, Miami Silt Loam from Indiana, Carlisle Muck from Michigan, and Carrington Loam from Nebraska. Thus despite somewhat limited records as to its occurrence, it seems probable that this organism is of worldwide distribution.

Stachybotrys, particularly *S. atra* Corda, has long been known to occur here under conditions similar to those described above. *S. atra* has been used to a limited extent during the past several years as a test organism for evaluating mildew resistance of fabrics.

In reviewing the genus *Stachybotrys*, Bisby (1) places *M. echinata* under the tentatively emended *S. subsimplex* Cooke as similar to the latter species, "except in having catenulate spores," suggesting that a reduction in slime production would allow for the retention of spores in chains. The rarity of *M. echinata* led him to view this species with suspicion. In accepting the species *S. subsimplex*, Bisby bases his conclusions on dried specimens, lost cultures, and prepared slides. The distinguishing feature of this

species is that the phialophores are "generally simple" in contrast to the branched phialophores of *S. atra*. In tentatively disposing of *M. echinata*, Bisby suggests that it may be "an unusual or abnormal development of *Stachybotrys*."

During the course of isolating and purifying cultures of so-called *M. echinata*, the writer observed four isolates of an intermediate nature between *Stachybotrys* and *Memnoniella*, as well as four isolates which, up to the present, have remained stable in the characters Galloway lists for *M. echinata*. No *Stachybotrys* that has been isolated or received in this laboratory falls into the description of *S. subsimplex* supplied by Bisby. However, the *Stachybotrys*-like phase of the intermediate isolates is much like the description of *S. subsimplex*.

CULTURES AND METHODS

Four cultures of *Memnoniella*, Serial Nos. 2, 52, 85, and 86, were examined. These had been secured from the NDRC Tropical Fungus Culture Collection at Harvard, having been sent to Dr. Wm. H. Weston through the courtesy of the Mycological Panel of the Australian Scientific Liaison Bureau, originally isolated from deteriorated material from the Southwest Pacific. Nos. 2, 52, and 86 were found producing the elliptical to oval, roughened, black spores in slimy heads as in *Stachybotrys*, as well as the typical, spherical, roughened, catenulate black spores of *Memnoniella*. Culture 85 revealed only typical *Memnoniella* spores in chains. Culture 2 produced only an occasional *Stachybotrys* type of head.

A culture isolated in this laboratory from cotton duck showing typical *M. echinata* under the microscope, numbered M64, also produced in culture both types of spores in slimy heads and long chains. The mildewed duck was submitted by the Philadelphia Navy Yard. An isolate, designated M92, made by the writer from a cotton duck strip from soil contact in compost continues, however, to resemble *M. echinata* as originally described and illustrated.

Two cultures, numbered 1229.2 and 1331.1, isolated eight years ago in this laboratory and maintained to the present under the designations of *Stachybotrys* and *Stachybotrys papyrogena*, re-

spectively, when revived this year were found to be typical *M. echinata*. The note made at the time of isolation and identification indicates that the culture produced black spores in chains. Thus it appears that these cultures were mislabeled, rather than that they have changed.

Single spore isolates of all cultures except 1229.2 and 1331.1 were made using a micromanipulator and placing the spores on a cotton extract agar described later. All cultures which exhibited the two spore types gave rise in single spore culture to the same intermediate type, whether from the oval *Stachybotrys*-like spore or the spherical *Memnoniella*-like spore. The two cultures which showed only the *Memnoniella* phase continue to do so in single spore cultures. Cultures 1229.2 and 1331.1 are from single-spore isolates originally.

The medium used consisted of an extract of 10 grams of ginned cotton fibers in 100 ml. of distilled water. The fibers are allowed to become thoroughly wet and then squeezed several times until most of the water is removed. This is then made up with C.P. chemicals as follows: 2.22 grams $MgSO_4.7H_2O$, 2.09 grams K_2HPO_4, 2.86 grams KH_2PO_4, and 3.00 grams NH_4NO_3, and 1 per cent dextrose. Owing to retention of some of the distilled water by the cotton fibers after squeezing, the volume is restored with distilled water. Two per cent agar was used. The medium is then sterilized at 15 lbs. for 15 minutes. Robbins and Ma (3) and Robbins and Schmitt (4) have shown cotton fibers to contain at least vitamins B_1, B_6, biotin, and an unidentified organic acid.

Growth on the medium described above was abundant. All observations and photomicrographs were made from material grown on this medium. Growth was at room temperatures and in the light and dark of day and night.

Incidentally, a rapid method for purifying cultures of *Memnoniella* of both bacterial and fungal contaminants used in the course of this work may be of interest to other workers. Strips of gray duck (not *bleached* duck) ½ inch in width and four inches long were placed in test tubes, the tubes plugged, and sterilized in the autoclave at 15 lbs. pressure for 15 minutes. These strips then were inoculated with pieces of agar containing the contami-

nated culture. The inoculated tubes were then placed in tightly closed Mason jars with about an inch of water in the bottom. Incubation was at room temperature. After a period of three or four weeks, the *Memnoniella* sporulates abundantly, with the phialophores on both sides of and at right angles to the substratum, thus facilitating the removal of spores with a needle. The moisture content of the cloth is apparently too low to permit the development of bacteria and many types of contaminating fungi that require mono- or disaccharides are automatically eliminated.

OBSERVATIONS

The *Stachybotrys*-like phase of the intermediate cultures is most conspicuous at the advancing edge of a colony. As the colony becomes older, the *Memnoniella* phase predominates and the cultures might be considered pure *Memnoniella*, unless carefully scrutinized from several mounts. The colonies are typically *Memnoniella* in general characters, being buff to almost salmon-pink (on the reverse) with sporulation most abundant in the center and spreading outward.

Figure 1A shows the typical *M. echinata* type of spores in chains. This is from culture M64. Also from culture M64 is the condition shown in figure 2, where the same hypha gives rise to a *Stachybotrys*-like head of oval spores and to a *Memnoniella*-like head of catenulate, rounded spores. Both phases, as well as branching of the phialophore, are shown in figure 1B, from culture SN86.

Although no photomicrograph is shown, chains of *Memnoniella* spores arising from a head of phialides have been observed surmounted by a cluster of the oval spores of the *Stachybotrys*-like phase. This is particularly so in culture M64. Apparently the same head of phialides can produce both spore types.

A preliminary experiment, using M64 as a source of inoculum, was conducted in which spores were dusted on a sterile unsized

FIG. 1. A, *Memnoniella* phase of culture M64; B, branched phialophore bearing Stachybotrys-like spores at right and *Memnoniella*-like spores on the vertical branch. From culture SN86. In Patterson's Mounting medium with erythrosin, after fixing of spores in chains with ½ ethanol 95½ glacial acetic acid. (Photomicrographs by M. L. Jaeger) × 1100.

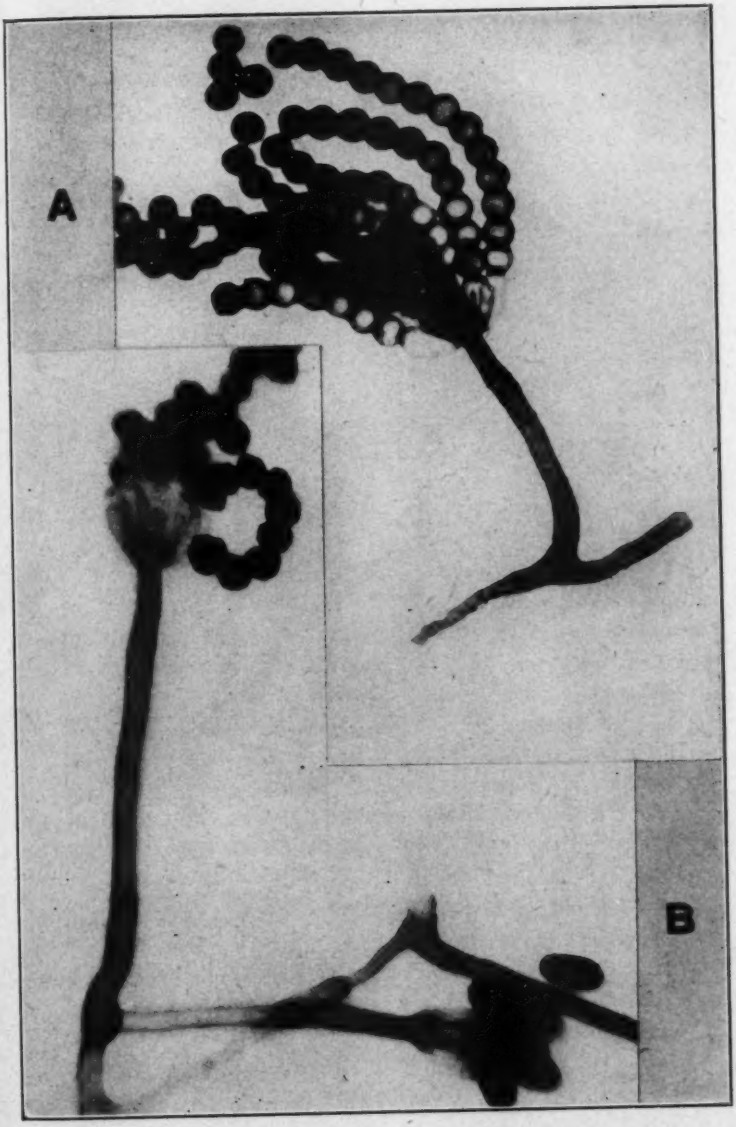

FIG. 1.

gray cotton duck strip in a test tube. This tube was placed in a sealed Mason jar with an inch of water and incubated for 6 weeks at 86° F. in the dark. The inoculum contained both spore types. Although sporulation was abundant, microscopic examination failed to reveal the two phases. Only the *Memnoniella* phase

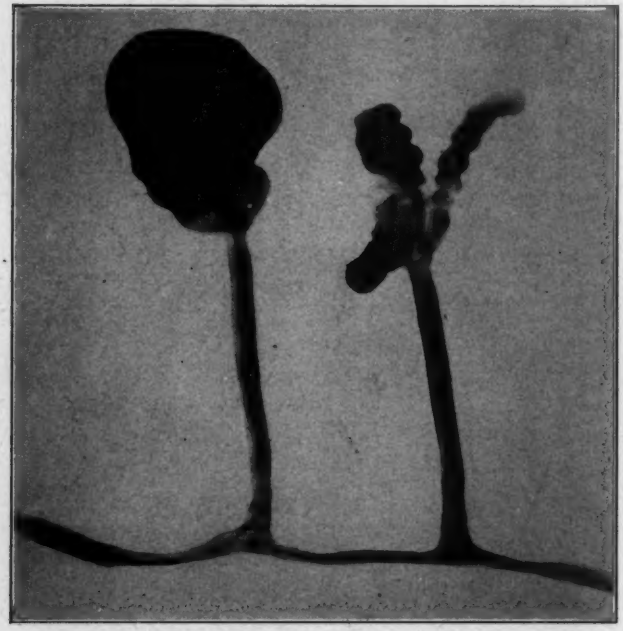

FIG. 2. *Memnoniella* phase at right with *Stachybotrys* phase at left on the same hypha, from culture M64. Preparation the same as for Fig. 1. (Photomicrograph by M. L. Jaeger) × 1100.

could be found. The tendency to produce both phases, then, is also governed by environmental conditions.

Permanent Clearcol mounts of each single-spore isolate culture have been made. The cultures are being maintained at the present.

DISCUSSION

The condition, in which two phases occur on the same hypha or branched phialophore, suggests that *Memnoniella* and *Stachy-*

botrys may be segregates from the intermediate type, or that the intermediate type is a hybrid resulting from a perfect stage yet to be discovered. Whatever the explanation, the demarcation between *Stachybotrys* and *Memnoniella* is rendered less clear by these isolates. The fact that Galloway's (2) isolate, from which the combination of names was made by him, upon being examined by Bisby ten years after isolation, continues as pure *Memnoniella echinata,* would indicate, along with the two eight-year-old isolates in this laboratory, that *M. echinata* is a valid entity. Likewise, the recognition by G. Smith (5) of *S. atra,* as well as by Bisby, would indicate it to be a valid, stable species. However, the scant description by Bisby, the loss of cultures, and the inability of the present writer to find a culture corresponding to *S. subsimplex*—unless this designation may be applied to the intermediate phase described in this paper—would seem to raise some question as to the validity of the species.

The erection of a specific name for the intermediate phase is probably of questionable value.

Should further study reveal perfect stages, perhaps the intermediate phase could be demonstrated experimentally. Until such an eventuality, however, it seems sufficient to call attention to this phenomenon in order that experimental and taxonomic workers may be aware that this condition exists.

SUMMARY

1. Isolates of an intermediate nature between *Memnoniella* von Höhnel and *Stachybotrys* Corda have been observed.

2. *Stachybotrys*-like slimy heads of elliptical to oval spores and *Memnoniella*-like heads of chains of spherical spores can occur on separate phialophores on the same hypha, or separately on the two phialophores of a branched phialophore.

3. Single-spore isolates of cultures exhibiting this phenomenon continue to do so, whether the single spore is of the elliptical *Stachybotrys* type or of the spherical *Memnoniella* type.

4. Isolates stable for the characters of *Memnoniella echinata* for eight years are also reported.

5. The form genera *Memnoniella* and *Stachybotrys* are considered to be valid entities.

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STUDIES IN THE GASTEROMYCETES XII. FIVE SPECIES OF TYLOSTOMA WITH MEMBRANOUS EXOPERIDIA

W. H. LONG

(WITH 5 FIGURES)

In a study of several thousand specimens of *Tylostoma,* including numerous species, I was surprised to find that only five species among all these plants had typical membranous exoperidia, while a study of the literature on this genus does not indicate any additional species with such exoperidia. The results of my studies are embodied in this paper. It is interesting to note that the five species are divided among the three mouth sections as follows: 2 in the fibrillose section, 2 in the tubular, and 1 in the indefinite mouth group.

The five species with membranous exoperidia are as follows: *Tylostoma poculatum* White, *T. Lloydii* Bresadola, *T. involucratum* Long, and two new species described in this paper.

TYLOSTOMA POCULATUM White, Bull. Torr. Club **28**: 431. 1901.
Tylostoma minutum White, Bull. Torr. Club **28**: 430. 1901.

Sporophore consisting of sporocarp, stipe and a slightly enlarged base, originating from 2 to 3 cm. below the surface of the soil. *Sporocarp* subglobose to slightly depressed-globose, 4–15 mm. high by 7–20 mm. wide, usually attached firmly to stem apex, but easily breaking off in or near the socket. *Exoperidium* strongly and permanently membranous, drying into a fragile envelope, early deciduous in pieces, usually leaving lacerate fragments of the dried membrane around the top of the peridial sheath, inner surface tilleul buff (Ridgway). *Peridial sheath* persistent, a thick band of agglutinated hyphae and sand, 2–7 mm. broad, often with a somewhat cup-shaped upper margin composed of the fragments of the exoperidium. *Endoperidium* perfectly smooth, usual color tilleul buff, but sometimes when freshly emerged vinaceous buff to fawn color, membranous. *Mouth* fibrillose, often seated in a distinct depression (FIG. 1), usually circular, but often elliptical,

mouth opening 1–2 mm. in diameter, surrounded by a definite fibrillose mat, 2–4 mm. across, elevated when young, but becoming nearly plane in age, concolorous with the endoperidium, but sometimes slightly darker. *Collar* inconspicuous, close to stem, 1–2 mm. distant. *Stipe* 1–3 cm. long by 2–3 mm. thick, even, terete, often striate above, white when freshly emerged but turning light brown (wood brown) on weathering, walls thin and fragile, easily breaking especially near or in the socket, often with a spongy cushion of hyphae and sand extending for ⅔ of the distance up the stem (FIG. 1, bottom row); this cushion is big at base but tapers above, it easily falls off when dry leaving a white friable area on stem. *Volva* none. *Radicating base* rare, roots short (FIG. 1, 2nd row from bottom). *Base* slightly enlarged. *Gleba* cinnamon rufous. *Capillitium* hyaline, branched, 4–7 μ thick, ends square, lumen small, septa rare, some slightly swollen. *Spores* subglobose to oval, often irregular and angular, 4.2–6 μ in diameter, rarely 7 μ, usual size 5.6 μ, some shortly pedicelled. *Epispore* ferruginose, smooth to verruculose.

The color of the endoperidium of the type of *T. poculatum* is a tilleul buff and not fawn color as given by White (l.c.). This is the usual color for this species.

ILLUSTRATIONS: White, the Tylostomaceae, Bull. Torr. Club **25**: *pl. 34, figs. 4–6.* Lloyd, Myc. Writ. **2**: *pl. 83, fig. 2, fig. 3* as *T. tuberculatum* and *figs. 4* and *5* as *T. subfuscum.*

HABITAT: Solitary, gregarious or rarely caespitose, in sand or sometimes in black soil, in open or in partial shade of mesquite brush (*Prosopis juliflora*), or other desert shrubs.

DISTRIBUTION: NORTH AMERICA. ARIZONA. SANTA CRUZ COUNTY, 6–8 miles from Nogales on Highway 89, elevation 3857 feet, *W. H. Long and Victor O. Sandberg,* February 19, 1934, *7625* (13 plants), June 4, 1938, *8305* (6 plants). *W. H. Long and David J. Stouffer,* September 11, 1941, *9630* (6 plants), *9631* (14 plants). PIMA COUNTY, 8 miles from Tucson on road to Sabino Canyon, elevation 2400 feet, *W. H. Long and Victor O. Sandberg,* September 22, 1934, *8019* (6 plants). COCONINO COUNTY, Kaibab Lodge, elevation 9800 feet, *W. H. Long,* October 22, 1933, *7881* (48 plants). Flagstaff, elevation 6894 feet, *W. H. Long,* May 6, 1934, *7693* (62 plants). YAVAPAI COUNTY, Prescott, elevation 6000 feet, *W. H. Long,* October 15, 1933, *7755* (10 plants). *Victor O. Sandberg and Carl Butler,* September 8,

1934, *7955* (4 plants). *Victor O. Sandberg,* January 2, 1934, *7905* (32 plants).

NEW MEXICO. SOCORRO COUNTY, Tres Montosos Mts. near Magdalena, elevation 6700 feet, *E. A. Frazier,* October 11, 1934, *7990* (5 plants). DONA ANA COUNTY, Jornado Experimental Range, elevation 4150 feet, *Ivan H. Crowell, W. H. Long and Victor O. Sandberg,* May 2, 1937, *8181* (4 plants). *W. H. Long,* November 12, 1938, *8259* (3 plants), *10032* (1 plant), October 2, 1939, *8404* (4 plants). *W. H. Long and David J. Stouffer,* September 7, 1941, *9589* (59 plants), September 8, 1941, *9604* (15 plants), *9611* (19 plants), *9707* (5 plants). VALENCIA COUNTY, east of Rio Grande River, 4 miles below Belen bridge, elevation 4785 feet, *W. H. Long,* September 18, 1941, *9682* (8 plants), September 24, 1941, *7919* (7 plants). *W. H. Long and David J. Stouffer,* December 6, 1941, *9927* (2 plants). LUNA COUNTY, 10 miles west of Deming, along highway 70, elevation 4300 feet, *W. H. Long and David J. Stouffer,* September 12, 1941, *9643* (11 plants), September 13, 1941, *10009* (11 plants). SANDOVAL COUNTY, 2 miles south of Bernalillo highway 85, elevation 5100 feet, *W. H. Long,* July 12, 1941, *9439* (11 plants). 4 miles east of San Ysidro, near state highway 84, elevation 6000 feet, July 12, 1941, *9408* (3 plants). 5 miles west of San Ysidro near state highway 84, elevation 6200 feet, July 9, 1941, *9385* (1 plant). 6 miles from Cuba, near Eureka Lodge in Jemez Mts., elevation 8300 feet, *W. H. Long,* October 13, 1933, *7777* (4 plants). CHAVEZ COUNTY, in oak shinnery (*Quercus harvardii*), 34 miles east of Roswell on highway 380, elevation 3400 feet, *W. H. Long and David J. Stouffer,* April 19, 1942, *10042* (48 plants). LINCOLN COUNTY, 8 miles south of Oscuro, elevation 5000 feet, *W. H. Long and David J. Stouffer,* April 18, 1942, *10092* (1 plant). Juniper sand hill area 25 miles west of Corona, elevation 7000 feet, *David J. Stouffer,* December 15, 1941, *9972* (3 plants), September, 1941, *9847* (4 plants). *W. H. Long and David J. Stouffer,* September 17, 1941, *9746* (4 plants). Pinos Mts. Area, elevation 7000 feet, *David J. Stouffer,* November 27, 1940, *9296* (2 plants). Jicarilla, elevation 6560 feet, *David J. Stouffer,* October 31, 1941, *9864* (2 plants). In vicinity of Corona, elevation 6500–7200 feet, *David J. Stouffer,* April 21, 1940, *7830* (49

plants), July 1941, *9530* (5 plants), June 2, 1941, *9426* (2 plants), July 23, 1941, *9414* (3 plants). *W. H. Long and David J. Stouffer,* April 21, 1941, *11042* (9 plants), September 17, 1941, *9789* (14 plants), September 15, 1941, *9697* (2 plants), September 6, 1941, *9575* (7 plants). Mescalero-Apache Indian Reservation, in White Mt. area, elevation 6560 feet, *Elmo Traylor,* October 15, 1941, *9934* (8 plants). TORRENCE COUNTY, 8 miles S.W. of Vaughn, elevation 5950 feet, *David J. Stouffer,* June 15, 1941, *9418* (6 plants).

TEXAS. DENTON COUNTY, Denton, elevation 620 feet, *W. H. Long 2062* (3 plants) in Lloyd Myc. Coll. *30697.*

NEBRASKA. BROWN COUNTY, Long Pine, February 3, 1896, *J. M. Bates 351* (3 plants) in Lloyd Myc. Coll. *33642.* CUSTER COUNTY, Calloway, *J. M. Bates,* November 11, 1902, Bates *2689* (20 plants) in Lloyd Myc. Coll. *33644. J. M. Bates,* December 9, 1902, from Ell. & Ev. Fungi Columb. *1889* (3 plants) in New York Botanical Herbarium. Oconto, *J. M. Bates,* September 30, 1902 (12 plants) in Lloyd Myc. Coll. *30830* as *T. subfuscum.*

WASHINGTON. YAKIMA COUNTY, Sunnyside, *J. S. Cotton,* comm. *C. V. Piper,* June 1900, Fungi of Washington, *739* (4 plants), in Lloyd Myc. Coll. *30834* as *T. tuberculatum.*

AUSTRALIA. *F. M. Reader,* comm. *D. McAlpine,* in Lloyd Myc. Coll. *30827* (9 plants). 4 of these plants are shown in Myc. Writ. **2**: plate 83, *fig. 4* as *T. subfuscum.*

SOUTH AUSTRALIA. ADELAIDE COUNTY, Adelaide. *J. Burton Cleland* (*751*) in Lloyd Myc. Coll. *30869* (5 plants).

NEW ZEALAND. WELLINGTON COUNTY, Wellington. *G. H. Cunningham* (*67*), September 1919, in Lloyd Myc. Coll. *50740* (40 plants).

"Lone Pine," Nebraska, given by Miss White as type locality for *T. poculatum* is incorrect, it should be spelled "Long Pine."

Fifty American collections of *Tylostoma poculatum* containing 544 plants were studied, each collection identical in every detail as to external characters. All of the collections had membranous exoperidia and were typical in size, shape and color for *T. poculatum.*

These collections ranged from Texas on the south to the state of Washington on the north, and from 620 feet elevation to 9800 feet,

from semidesert areas in Arizona and New Mexico, to less arid conditions in Texas, northern Arizona and New Mexico and in the state of Washington. Some were from areas where snow does not occur, others were found where the winters were severe with a snow fall of 3–4 feet. The soil was usually sandy, but 3 collections came from old stock corrals with rich soil and 2 from a loamy, rich, black soil. This great range in habitat is shown in detail under "Distribution" for each collection.

Forty-four of these collections were examined with a compound microscope to determine the glebal characters, especially the size, shape and markings of the spores for each collection. Especial effort was made to determine whether each had smooth or rough spores.

The microscopic examination gave the following results: 12 of the collections showed verruculose spores, 12 gave a mixture of verruculose and smooth spores on the same slide, while 22 of the collections gave only smooth spores, yet all of these collections were undoubtedly the same species in spite of the spore variation as to markings. 1 to 3 sporocarps in each collection were examined under the microscope since this was considered sufficient to get the characteristics of the spores. In the collection from the 9800 feet elevation, 3 separate sporocarps were examined, 2 gave verruculose spores while 1 gave both verruculose and smooth spores.

In view of the above data the spore markings of *T. poculatum* should be listed as smooth to verruculose and not just smooth. I cannot see any valid reason why a species should not have minor variations in spore markings as well as in their shape, size, etc. Also we have minor variations in macroscopic characters such as size and color within narrow limits of sporocarp and stem, but the membranous exoperidia is a fixed character for a given species and not subject to variation.

I have examined the type material of both *Tylostoma poculatum* and *T. minutum* and find that they are unquestionably the same species. In the type of *T. minutum,* I found only a few verruculose spores, most of the spores being smooth.

Figure 1 shows 25 specimens (natural size) of *T. poculatum* taken from 20 different collections. The bottom row shows 5

plants with spongy cushion bases, each plant from a different collection, ranging from the hot arid sand dunes of New Mexico to the cold regions of higher altitudes. The 2 plants with "black" stems came from 9800 and 8300 feet elevation respectively, while the other 3 plants in this bottom row were from lower elevations. One plant has the cushion scraped off to show the white stem inside the cushion. The 2nd row of plants from the bottom shows 4 plants with radicating roots. The upper 2 rows show the various sizes of plants, and characters of stems and heads. Several of the plants show the slight depression in which the peristome is often situated.

The utter futility of attempting to differentiate between species by their spore characters alone without taking into consideration their external characters is well illustrated by Cunningham (**1942**) in his discussion of *Tylostoma obesum* where he lists as synonyms the following species: *T. poculatum, T. gracile, T. kansense* and *T. Lloydii*. Even a layman can see from a casual comparison of figures 1, 2, and 3 that the 3 plants are as diverse in all their macroscopic characters as 3 species of *Tylostoma* could possibly be. They do not have even a superficial resemblance to each other and yet according to Cunningham they are the same. *T. poculatum* and *T. Lloydii* have membranous exoperidia, but the American version of *T. obesum* does not. *T. kansense* does not have a membranous exoperidium and has an entirely different mouth from any of the 3 others. Its mouth is indefinite and lacerate, it is also a white plant having both stem and endoperidium white. Now as to *T. gracile*, I have before me a box from the New York Botanical Garden marked *"Type of Tylostoma gracile White."* It contains a small specimen of *Chlamydopus meyenianus* and nothing else so apparently *"T. gracile"* was based on this specimen. This does not seem possible and yet this is the only authentic material of *"T. gracile"* known to exist. Furthermore the original *T. obesum* as published by Cooke & Ellis (**1878**) in Grevillea has a *tubular* mouth judging from the original description and figures, and it is so listed by Saccardo (**1888**) in Sylloge Fungorum, being placed under the *Eutylostoma* or tubular section. The American plant supposed to be the co-type is quite a different plant having a fibrillose mouth and differing in external charac-

ters from the plant described by Cooke & Ellis. Miss White (l.c.) was worried by these discrepancies, but passed them off by suggesting that the Cooke & Ellis plants were probably immature, though how this could change a tubular mouth to a fibrillose one is hard to understand. The statement that the American plant which Ellis retained when he sent the others to England is the same species does not make it true. It is not uncommon to find more than 1 species of *Tylostoma* mixed in a given collection. The determining factor in assigning the American plant to *T. obesum* was the similarity of the spore characters and such similarity cannot be depended upon for identification purposes, as is well shown in *T. poculatum, T. Lloydii* and *T. obesum* (American version). What our American plant described and figured by White and Lloyd is I do not know, possibly an undescribed species.

I find that spore markings are very unsatisfactory criteria for differentiating many species, especially those from arid regions, since most of them have very similar spores and yet the external characters are so different that they could not be the same species. That you can determine a species of *Tylostoma* from the spore markings alone is a myth, as far as my specimens are concerned.

I usually make 3 separate mounts from each sporocarp. 1 in water, 1 in chloral hydrate and 1 in benzoazurine. The water mount gives the true colors of spores and capillitium and usually the markings of the epispore. My findings are then checked in chloral hydrate and benzoazurine mounts. An oil immersion objective is used for all spore measurements and panchromatic films with the camera stopped down to 64 U. S. are used for making the photographs.

TYLOSTOMA LLOYDII Bresadola in Petri, Ann. Myc. 2: 423. 1904. FIG. 2.

Sporophore consisting of sporocarp, stipe and bulbous base, originating 1½–3 cm. below the surface of the leaf debris but still in the debris. *Sporocarp* suglobose, 5–8 mm. high by 7–12 mm. wide, firmly attached to the stem apex. *Exoperidium* permanently membranous, early deciduous in pieces or flakes. *Peridial sheath* persistent, membranous, a thin laciniate narrow, irregular band around base of sporocarp, margined with white, walnut brown, 3–5 mm. broad. *Endoperidium* papyraceous, perfectly

smooth, walnut brown to pecan brown. *Mouth* fibrillose, often seated in a decided circular depression, an irregular circular orifice, 1–2 mm. in diameter, peristome white, 3–4 mm. across, plane in material at hand. *Collar* definite, irregularly laciniate, projecting around stem, about 1 mm. long and 1–1½ mm. distant from stem. *Stipe* slender, terete, tapering toward apex, more or less curved, 4–8 cm. tall by 2–3 mm. thick at apex and 4–5 mm. at base, with appressed brown scales (Roods brown), more or less deciduous, leaving the stem mikado brown; surrounded at the base with a volvoid structure consisting of the lacerate membranous fragments of the upper part of the cortex, dark brown (Roods brown), often reflexed, 2–3 mm. high. *Bulb* a mass of white mycelium interwoven in the leaf debris, 10–15 mm. across. *Gleba* cinnamon color. *Capillitium* hyaline, thick walled, irregular, 3–7 μ thick, septa rare, slightly swollen. *Spores* 3.5–4 μ diameter. *Epispore* smooth.

HABITAT: gregarious or caespitose under shade of trees in thick leaf debris.

DISTRIBUTION: OHIO near Cincinnati, *Prof. W. H. Aiken,* in Lloyd Myc. Coll. *4498* (*7* plants); also in herbarium of Bresadola.

ILLUSTRATIONS: Bresadola in Petri. Ann. Myc. **2**: *pl. 6, fig. 4,* and 1 text figure; Lloyd Myc. Writ. **2**: *pl. 82, figs. 5–8.*

Bresadola (**1904**) states the mouth is oblong, 1½ × 2 mm., slightly protruding. Lloyd (**1906**) says the mouth is at first raised, shield-shaped, fibrillose. In the *7* specimens at hand the mouths must be old as they are not oblong, but irregularly orbicular and plane, having traces of the shield-shaped fibrillose mouths mentioned by Lloyd. The sub-cinereous appearance mentioned by Bresadola is found on several of the sporocarps especially just above the peridial sheath, but on only a portion of these heads while the general color is brown. This is the only species of *Tylostoma* known to me that has a white fibrillose peristome.

TYLOSTOMA INVOLUCRATUM Long, Mycologia **36**: 330–332. 1944.

This species was recently described by me (**1944**), hence it is not necessary to repeat the data given then; however, additional facts on distribution have since been obtained and these are given here.

DISTRIBUTION: CALIFORNIA. SAN BERNARDINO COUNTY, San Bernardino, elevation 1080 feet, *S. B. Parish,* Lloyd Myc. Coll.

53170 (3 plants) and Lloyd *52541* (1 plant). COLORADO. EL PASO COUNTY, Colorado Springs, elevation 5900 feet, *F. K. Vreeland,* Lloyd Myc. Coll. *53156* (3 plants). ARIZONA. PIMA COUNTY, near Tucson, elevation 2400 feet, *David Griffith (2239)*, January 1901, Lloyd Myc. Coll. *34491* (2 plants); Papago Indian Reservation, Santa Rosa Ranch, Children's Shrine, elevation 2300 feet, July 10, 1942, *Paul C. Lightle, 10265* (3 plants) in Long Herb. NEW MEXICO. SAN JUAN COUNTY, Chaco Canyon, elevation 6233 feet, *F. K. Vreeland,* in Lloyd Myc. Coll. *53165* (1 plant).

This extends the range of this species to 4 states, Arizona, California, Colorado and New Mexico.

Tylostoma xerophilum sp. nov. FIG. 5.

Sporocarpo globoso usque depresso-globoso, 4–5 mm. alto, 6–8 mm. lato. *Exoperidio* membranaceo, brunneo, toto secedente. *Endoperidio* toto levi, papyraceo, albo. *Ore* regulari integro, prominenti, mammoso, minuto. *Stipite* tenui, 15–25 mm. alto, 2–3 mm. crasso, brunneo. *Sporis* subglobosis, 4–5μ. *Episporio* granuloso, fulvo.

Sporophore consisting of sporocarp and stipe. *Sporocarp* subglobose to depressed-globose, 4–5 mm. high by 6–8 mm. wide, firmly attached to stem apex. *Exoperidium* strongly and permanently membranous, outer surface walnut brown covered with a sandy layer, inner surface white, drying to a thin, very fragile membrane deciduous in flakes, leaving lacerate shreds of dried membrane around top of the peridial sheath. *Peridial sheath* a thin membranous band under the head, 3–4 mm. wide, with an irregular border, semi-deciduous. *Endoperidium* white, perfectly smooth, papyraceous. *Mouth* tubular, small, circular, prominent. *Collar* inconspicuous, close to stem. *Stipe* very slender, weak, easily breaking especially near base, straight, uniform, 1½–2½ cm. long by 2–3 mm. thick, often striate on part above ground, smooth below, pecan brown. *Volva* none. *Radicating base* none. *Gleba* cinnamon color. *Capillitium* hyaline, same diameter as spores, septa slightly swollen. *Spores* subglobose, 4–5 μ in diameter. *Epispore* sayal brown, minutely verruculose.

HABITAT: Solitary or in small groups in partial shade, in mesquite-catclaw flats (*Prosopis-Acacia*).

DISTRIBUTION: ARIZONA, SANTA CRUZ COUNTY, 7 miles from Nogales on state highway 89, elevation 3857 feet, *W. H. Long and Victor O. Sandberg,* November 13, 1936, *8847* (7 plants). *W. H.*

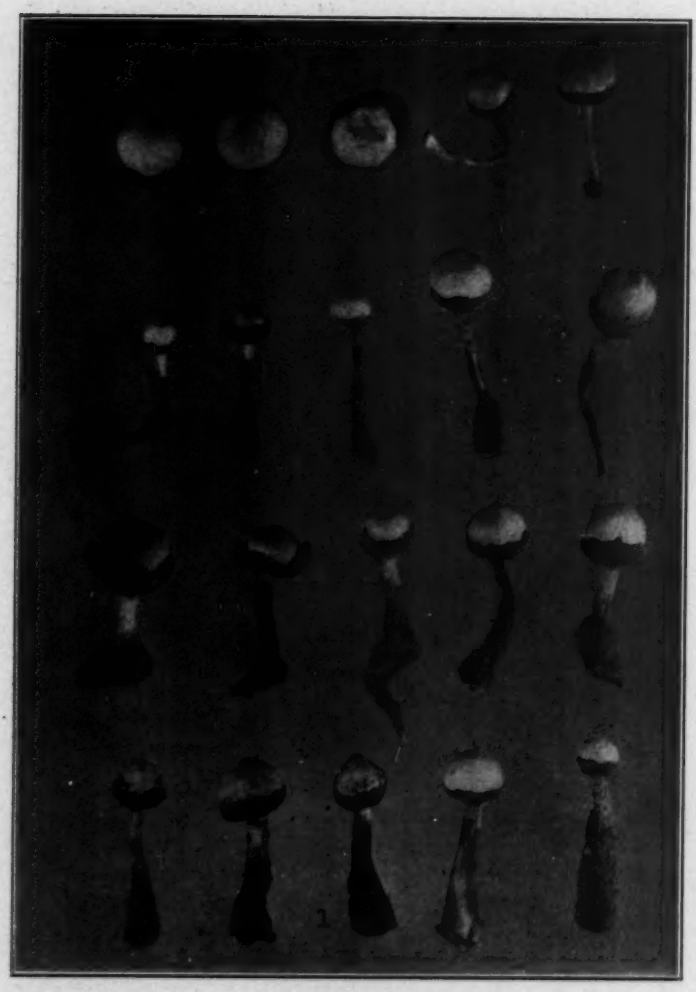

FIG. 1. *Tylostoma poculatum* × 1.

Long and David J. Stouffer, September 11, 1941, *9688* Type (7 plants).

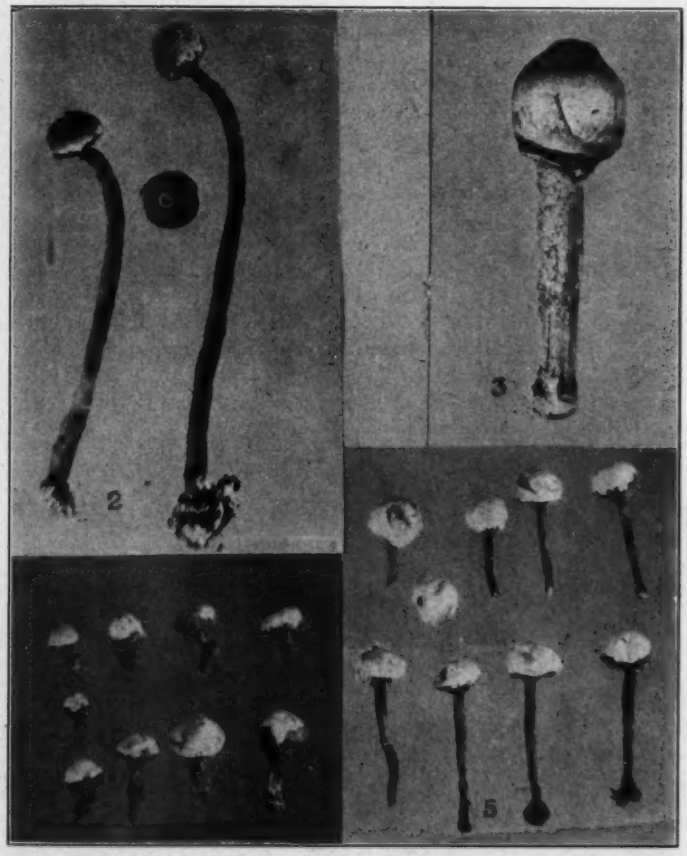

FIG. 2. *Tylostoma Lloydii;* 3, *T. obesum*—American version; 4, *T. parvissimum;* 5, *T. xerophilum* all × 1.

Tylostoma parvissimum Long & Ahmad sp. nov. FIG. 4.

Sporophora parvissima. *Sporocarpo* ovato usque subgloboso 5–8 mm. alto 5–10 mm. lato. *Exoperidium* membranaceo, albo, secedente. *Endoperidio* toto levi, membranaceo, albo. *Ore* indefinito, plano usque parum prominenti. *Stipite* tenui, 2–10 mm. alto 1–2 mm. crasso, brunneo. *Sporis* globosis usque subglobosis, 4.2–5.4 μ. *Episporio* parum echinulato, hyalino usque parum colorato.

Sporophore consisting of sporocarp, stipe and radicating base. *Sporocarp* ovate to subglobose, 5–8 mm. high by 5–10 mm. in diameter, firmly attached to stem apex. *Exoperidium* strongly and permanently membranous, outer surface covered with an adnate layer of dry clay, white under clay, slowly deciduous in flakes, leaving lacerate shreds of the dried membrane on spore sac. *Peridial sheath* a band of agglutinated hyphae and clay, persistent, 2–3 mm. broad. *Endoperidium* white, smooth, tough, membranous. *Mouth* indefinite, slightly raised, becoming a small irregular ellipsoid to orbicular orifice, about 1 mm. wide, naked with no signs of fibrils. *Collar* 1–2 mm. long, conspicuous, closely clasping the stipe, covered with dried clay. *Stipe* slender, 2–10 mm. tall by 1–2 mm. thick (exclusive of root), uniform, light brown (wood brown under the layer of clay), smooth to faintly longitudinally striate. *Volva* none; *bulb* small, firm. *Radicating* base with one stout root, 4–10 mm. long. *Gleba* clay color to raw sienna. *Capillitium* hyaline, much branched, side branches much smaller, 2–6 mm. thick, lumen none, flexuous, ends of threads square, septa rare, not swollen. *Spores* globose to broadly oval, 1-guttulate in water mount, 4.2 to 5.4 μ, usual size 4.5 μ. *Epispore* hyaline to slightly fulvous, about 1 μ thick, appearing granular in water mount, but distinctly and moderately echinulate in chloral hydrate mount, also in benzoazurin mount.

HABITAT: in clayey soil, common on such areas; in semi-desert regions.

DISTRIBUTION: INDIA, Panjab Plains, Rohtak District, Rohtak. Fungi of the Panjab Plains (*338*). *S. Ahmad,* June 15, 1941 (10 plants), Long Herbarium *10621* Type. Herbarium Sultan Ahmad; Fungi of India (*338a*). *S. Ahmad,* July 1941 (6 plants) in Long Herbarium *11026.* This pretty little species is characterized by the permanently membranous exoperidium, the indefinite naked mouth, the echinulate spores, its miniature stature and by its clay habitat.

GENERAL REMARKS

It is almost impossible to determine the earlier species of *Tylostoma* from their original descriptions and one must, therefore, examine the type or authentic material to know what the species really is. Most of the earlier descriptions are very meager and omit most of the fundamental characters necessary for determination.

Line drawings also are practically worthless for identification purposes, since they usually do not show the real characters of the species. Likewise many of the earlier photographs are so poor that they do not show the principal characters of the species, although there are some outstanding examples of good photographs of *Tylostoma,* notably those published by Lloyd.

The habitat is often a very important character of a given species. It may grow in leaf debris in deep shade as do *Tylostoma Lloydii, T. simulans,* and *T. pygmaeum,* where the debris is held as a ball on the base of the stem; or the species may grow in sand in open unshaded areas as is the case with most species, or rarely a species may grow in a hard-pan clay soil as for example *T. kansense, T. meristostoma,* and *T. parvissimum.*

I have no apologies to offer for using Color Standards (Ridgway, 1912), since I find that the real color is of much importance. The readers can then check the chart to see just what color is meant, while if I say "reddish brown," "bay brown" etc., no one can identify such colors, as each person has his own ideas as to what such colors are; you cannot have stability by using such terms. The colors of the stipe and the endoperidium for any given species are very constant and are of much help in the identification of the species.

Below I am giving the spelling used by Persoon for the 9 new species I have described in this and previous papers. I do not believe in perpetuating an error even if it was made by Persoon. However, as some authors do and insist on following and thus continuing his error, I here list my species as Persoon, this will save future name jugglers some needless trouble: *Tulostoma cretaceum, Tulostoma lysocephalum, Tulostoma opacum, Tulostoma involucratum, Tulostoma excentricum, Tulostoma meristostoma, Tulostoma macrocephalum, Tulostoma parvissimum* and *Tulostoma xerophilum.*

ACKNOWLEDGMENTS

I am under many obligations to Mr. John A. Stevenson for loan of material and important advice; to Dr. Fred J. Seaver for loan of material; to Dr. David H. Linder for advice on the names and to Prof. Sultan Ahmad for valuable material from India.

ALBUQUERQUE, NEW MEXICO

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FACTORS AFFECTING THE PRODUCTION OF RESISTANT SPORANGIA OF ALLOMYCES ARBUSCULA

RICHARD C. JONES [1]

(WITH 1 FIGURE)

In the life cycle of *Allomyces* the resistant sporangium has been the object of increasingly great interest, because it is in this structure, on the asexual plant, that the crucial meiotic division occurs. Sorgel (**8**), Kneip (**7**), and Barrett (**1**) have made detailed studies of the morphology of the resistant sporangium in connection with their studies on the life cycle. Emerson (**2**) carried out extensive studies upon the variations in size of the resistant sporangia on the thirty-one strains of *Allomyces* with which he worked. Hatch (**4**) contributed to our understanding of this critical structure by working out the details of the zoosporogenesis which occurs within its relatively heavy walls. Hatch and Jones (**6**) have provided some evidence on the maturation period which appears to be necessary before the resistant sporangium can produce viable zoospores. All of the workers on this fungus have shown a keen interest in the nature of the products of the resistant sporangium, and many of them have reported upon occasional departures from the usual sexual condition of these products. In spite of all of these studies, there is still a great deal which we must learn about the resistant sporangium before we can thoroughly and confidently interpret it. Our knowledge is especially incomplete with respect to the physiology and cytology of the resistant sporangium.

One of the problems with which workers on *Allomyces* have had to contend has been the uncertainty of development of viable resistant sporangia in agar cultures through the summer months. In the summer of 1939, Hatch (**5**) and his students were delayed in their studies by an almost total lack of resistant sporangia in

[1] This study was carried out at the State College of Washington, Pullman, Washington.

agar cultures grown at the Dartmouth College laboratories in Hanover, New Hampshire. In 1942, and again in 1943, the same difficulty was encountered at the State College of Washington in Pullman, Washington. Because all of these failures in development were encountered during the late spring and summer months, and because no such difficulty has ever been reported for the winter months, it appeared that the failure of the resistant sporangia to develop might be traced to some seasonal factor which was present during the summer months. An analysis of seasonal conditions at Hanover and Pullman suggested that an explanation was most likely to be found in the photoperiod, because the seasonal variation of this factor appeared greater than the seasonal variation of any other factor effective within the laboratories. Accordingly, experiments were set up for the purpose of gaining any useful information on the effect of the photoperiod upon production of resistant sporangia in agar cultures of *Allomyces*. This problem was thought to be worthwhile for a second reason: that apparently no work has been done dealing with the influence of the photoperiod on growth and reproduction with the Phycomycetes.

EXPERIMENT I. The first experiment was set up on January 28, 1944. Four series, each consisting of ten asexual cultures in maltose-peptone agar plates, were prepared. Series I was grown in total darkness throughout the day and night, except for a few seconds daily when it was examined and its growth recorded. Series II was provided with sixteen hours of complete darkness and eight hours of daylight each day. Series III was treated with eight hours of darkness and sixteen hours of light—daylight supplemented with artificial light. Series IV received continuous light—daylight during the daytime and artificial lighting at night. Series I, II, and IV were set up in the same laboratory, but series III was grown in an adjoining laboratory. Series III was cultured apart from the other series in order to take advantage of a controlled sixteen-hour light day in the second laboratory. The door between the two laboratories was kept open at all times. A thermograph provided a record of the temperature, and thermometers, placed beside each series, were checked periodically against the thermograph readings. The growth for all four series

was recorded, and at the end of the experiment all of the cultures were examined for the presence of resistant sporangia.

In series III no increase in the diameter of the colonies could be detected from the seventh to the eleventh day. The terminal seg-

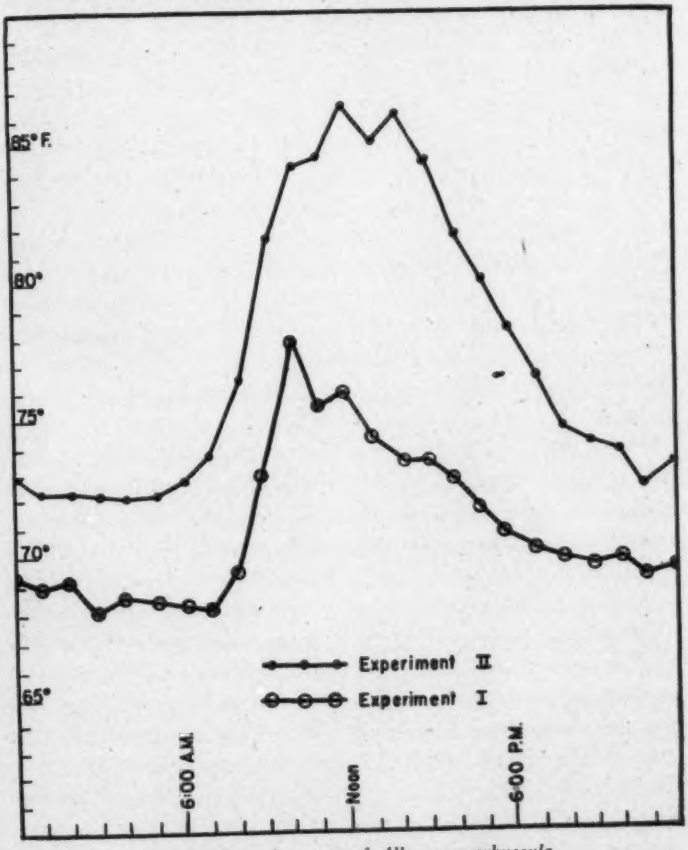

FIG. 1. Growth curves of *Allomyces arbuscula*.

ments of these hyphae had been seriously injured or killed, with a resulting plasmolysis. The new growth which had branched out behind these tips, however, appeared to have developed at a normal rate. Since series III had been placed in a different room from the other series, it was believed at the time that the factor

responsible for the interruption was probably to be found only in that room—possibly an excessively high light intensity, because the room was equipped with overhead windows, or possibly the fact that during the middle of sunny days series III experienced a slightly higher temperature than the other series.

Large numbers of resistant sporangia were produced throughout series I, II, and IV. In series III, however, although resistant sporangia developed in nearly comparable numbers up until the interruption in growth, only an occasional resistant sporangium could be found on mycelium which developed after the interruption. Moreover, although the resistant sporangia in series I, II, and IV were uniformly amber-yellow, the resistant sporangia throughout series III appeared to be of a paler color than those in any of the other series. Just what significance can be attributed to these observations it is difficult to say. Although photoperiod may have been a factor it is entirely possible that the differences in light intensity and/or temperature may also have exerted some influence.

EXPERIMENT II. In order to clarify the results of experiment I and to further explore the interruption of growth which occurred in series III, the experiment was repeated under more carefully controlled conditions. The second experiment began on March 17, 1944.

In this second experiment all four series were set up on the same table, situated in the same room where series III of the first experiment had been grown. In order to keep the temperatures more uniform for all cultures and to guard against too intense illumination (a possible cause for the temporary interruption of growth in series III of the first experiment), the plates were shielded from the direct rays of the sun by means of a screen placed between them and the windows. All four series were examined daily and their growth was recorded as before.

The most important difference in the growth of the plants was the almost total lack of resistant sporangia in experiment II. In only one of the forty plates (a plate exposed to twenty-four hours of light) and then only in a small sector, were any resistant sporangia found.

The failure of resistant sporangia to form in the second experiment is apparently not to be attributed to light intensity, because even though the intensity of daylight did differ somewhat during the two periods, it is to be noted that resistant sporangia failed to develop in those plates which were grown in total darkness quite as completely as they did in cultures which were exposed to different periods of light. The differences in the results of the two experiments cannot be attributed to humidity, for the relative humidity must have been very closely comparable in both experiments; the same kind and quantity of medium were used in both experiments, and the cultures were carried in the same type of culture dishes. The one factor which did vary, however, was temperature.

It appeared, then, that the differences in temperature offered a possible explanation for the faster growth and the lack of resistant sporangia in the second experiment. An examination of the thermograph charts revealed that the first experiment was run under generally lower temperatures than the second. The temperatures during the entire run ranged, in the first experiment, from a minimum of 60° F. to a maximum of 93° F. In the second experiment, the temperatures reached a minimum of 64° F. and a maximum of 103° F. Since the temperature curves varied from day to day during each experiment, and because these relatively small variations appeared to have had little, if any, appreciable effect on the growth and development of the fungus (the cultures were very uniform in their day-to-day growth), a study of the temperature curves for each day, individually, would serve no useful purpose. In order to determine the difference in temperature for an average day in each experiment, the mean hourly temperatures for the whole period were computed. The results appear in table 1.

Since minimum and maximum temperatures for each day did not always fall upon the same hour or, indeed, exactly upon any hour, the average daily minimum and maximum temperatures are here presented separately at the bottom of table 1. In experiment I, the average daily minimum was 67.5° F.; the average daily maximum was 80.3° F. In experiment II, the average daily minimum was 69.6° F.; the average daily maximum was 86.4° F.

If a temperature curve is now plotted for each experiment, based on the average hourly readings, and the two curves are drawn on the same graph, there results the condition illustrated in figure 1. It can be noted there that not only are the average hourly temperatures of the second experiment consistently higher than the

TABLE 1

AVERAGE HOURLY TEMPERATURE
(in Degrees Fahrenheit)

Hour	Experiment I	Experiment II
1:00 A.M.	69.2	72.3
2:00	69.3	72.4
3:00	68.3	72.4
4:00	68.8	72.2
5:00	68.6	72.2
6:00	68.6	72.4
7:00	68.4	73.5
8:00	69.5	76.2
9:00	73.4	81.5
10:00	78.1	84.3
11:00	75.4	84.6
12:00	76.0	86.4
1:00 P.M.	74.3	85.1
2:00	73.7	86.0
3:00	73.5	84.4
4:00	73.1	81.7
5:00	72.1	80.2
6:00	71.0	78.2
7:00	70.5	76.4
8:00	70.1	74.5
9:00	69.1	74.1
10:00	70.0	73.8
11:00	69.5	72.6
12:00	69.5	73.0
Average daily minimum	67.5	69.6
Average daily maximum	80.3	86.4

corresponding values in the first experiment, but the duration of the midday elevation is considerably longer, the proportion of relatively high temperatures being greater as a result of this condition. This, of course, reflects the fact that the sun's heat warmed the room for a longer period in late March than it did in late January and early February. It should be clearly evident that the total amount of heat to which the cultures of the second experiment were subjected was much greater than the amount experienced by the cultures in the first experiment. In summarization, therefore, it may be said that experiment II differed in its temperature relationships from experiment I in that the temperature attained a higher minimum and a higher maximum, that the warm

part of the day was relatively longer, and that the aggregate heat experienced by the cultures was much greater.

It cannot be said that because the minimum and maximum temperatures of any one day in the first experiment were above those of any single day in the second experiment, the minimum and maximum values are not critical. Such a conclusion would be unwarranted because although conditions might inhibit the formation of resistant sporangia on any single day, these structures might develop in the same area under more favorable conditions during the next two or three days. This is possible because resistant sporangia may continue to form for at least four days after the laying down of new vegetative growth. This fact is substantiated by the observation that in growing cultures, produced under normal conditions, resistant sporangia are known to be progressively more numerous as one proceeds from the outer perimeter of a colony inward through the four-day-old growth.

It appears, then, that in the culturing of *Allomyces arbusculus* higher temperatures, at least those within the range of these experiments, are not as conducive to the development of resistant sporangia as are the lower temperatures. What still remains to be established, however, is whether the failure of the cultures in the second experiment to develop resistant sporangia was due to the higher minimum temperature in that experiment, the higher maximum temperature, the longer period of relative high temperature, or the shorter duration of relatively low temperature. The fluctuation in the daily temperature was ruled out as a possible explanation of this problem because Hatch (3) observed in his work on zonation, that resistant sporangia developed regularly, even when the daily fluctuation in temperature did not exceed 0.5° C.

EXPERIMENT III. When the results of the two experiments were known, a third experiment was set up in order to learn whether the minimum and/or maximum temperatures which had occurred in the first two experiments were important factors in the development, or failure of development, of resistant sporangia.

Maltose-peptone agar cultures were prepared as before and placed in a constant temperature apparatus. One such apparatus was maintained at 71° F. The reason for selecting this tempera-

ture was to determine whether resistant sporangia would form at a constant (and therefore average minimum) temperature of 71° F. If resistant sporangia could be produced at an average minimum temperature of 71° F., then their failure to develop in experiment II (where the average minimum temperature was 69.6° F.) could not be attributed to an excessively high minimum temperature.

A second constant temperature apparatus was maintained at a temperature of 79° F. This temperature was selected in order to determine whether resistant sporangia would form at this relatively high constant (and therefore average maximum) temperature. If resistant sporangia should fail to develop at the average maximum temperature of 79° F., after having developed in experiment I (where the average maximum temperature was 80.3° F.), then failure to develop here could not be attributed to an excessively high maximum temperature.

Four sets of asexual cultures were set up as follows: Series I was grown at 71° F. throughout the experiment. Series II was subjected to 71° F. for sixteen hours of each day but was maintained at 79° F. for the other eight hours. Series III was exposed to 79° F. for sixteen hours of each day and to 71° F. for the remaining eight hours. Series IV was carried in the 79° F. temperature throughout the experiment. All cultures were grown at the same time, on the same batch of medium, and in total darkness.

The most significant results were those which concerned the production of resistant sporangia. On the same day in which experiment III was discontinued, all of the cultures were examined for the presence of resistant sporangia. Every plate was found to contain considerable numbers of these structures. However, series I (grown at 71° F.) seemed to have produced a comparatively heavy yield of these thick-walled bodies. This was especially noticeable when series I was compared with series IV which exhibited a relatively sparse distribution. In order to obtain comparative figures on the relative numbers of resistant sporangia in each series, counts were taken from representative plates.

The method used in making counts of resistant sporangia follows. From series I and IV, four plates each were chosen; from series II and III, two plates each were chosen. All plates were selected on the basis of their general healthy appearance. Each

plate was then placed on the stage of a microscope under a 10 mm. objective and the resistant sporangia within a single field were counted. Counts of this sort were made for ten different fields in each plate, and the average number of resistant sporangia per field was computed for each culture. At least two fields were selected from each quarter-segment of any given plate, in order to obtain figures which would be, a nearly as possible, representative of the plate as a whole. Care was also taken to select all fields from within the line marking four-day-old mycelium so that the resistant sporangia would have approximately attained their maximum number in each area studied. Aside from these two precautions all fields were chosen at random. The figures representing all counts appear in table 2.

TABLE 2

Series	Number of resistant sporangia											Plate average	Average for series
I	Plate 1	63	81	59	74	77	69	75	72	75	79	72.4	
24 hrs. at 71	Plate 2	80	78	66	71	75	78	70	69	72	78	73.6	
	Plate 3	70	74	79	64	63	72	78	72	63	68	70.3	72.27
	Plate 4	78	69	72	76	68	75	72	66	75	77	72.8	
II													
16 hrs. at 71	Plate 1	53	71	49	65	67	58	55	63	65	52	59.8	
8 hrs. at 79	Plate 2	56	63	51	53	54	59	64	62	60	59	58.1	58.9
III													
8 hrs. at 71	Plate 1	39	42	40	48	43	45	40	45	47	45	43.4	
16 hrs. at 79	Plate 2	36	45	48	42	49	49	51	43	38	40	44.1	43.75
IV	Plate 1	39	40	36	40	51	32	36	40	41	38	39.3	
24 hrs. at 79	Plate 2	34	42	38	36	38	35	40	39	42	41	38.5	
	Plate 3	41	39	32	37	36	36	34	42	41	36	37.4	38.25
	Plate 4	36	42	38	33	41	39	37	38	35	39	37.8	

Effect of high and low temperatures. An analysis of table 2 shows that more resistant sporangia were formed in series I (71° F.) than in series IV (79° F.), confirming our previous observations that lower temperatures are more favorable for the development of resistant sporangia.

Effect of minimum temperature. It should be noted that series I formed more resistant sporangia than did series II, and that series II produced more resistant sporangia than series III. This fact is significant because all three of these series had the same

minimum temperature (71° F.). It can therefore be stated with reasonable confidence that the differences in the number of resistant sporangia could not have resulted from differences in the absolute minimum temperatures. Moreover, since 71° F. is above the average minimum temperature which prevailed in experiment II, it appears likely that the failure of resistant sporangia to form in that experiment had not been due to an excessively high minimum temperature.

Effect of maximum temperature. A comparison of the figures obtained for series II, III, and IV reveals that series II produced more resistant sporangia than did series III, and that series III produced more resistant sporangia than series IV. When it is considered that all three of these series possessed the same maximum temperature (79° F.), it may be concluded that this gradual decline in the production of resistant sporangia could not have been caused by an excessively high maximum temperature. Since some resistant sporangia formed in series IV, it still cannot be definitely concluded that the failure of resistant sporangia to form in experiment II could not have been due in part to an excessively high maximum temperature. However, these experiments make it appear highly improbable that maximum temperature was effective to any great degree.

PERIODICITY OF HIGH AND LOW TEMPERATURE. A further examination of the figures for series I, II, and III shows a positive relationship between the increase in duration of the cool part of the day and the increase in number of resistant sporangia produced. Likewise, a comparison of the figures for series II, III, and IV reveals a gradual decrease in the number of resistant sporangia as the warm part of the day was lengthened. We may conclude that where these temperatures are concerned, a relatively long duration of low temperature tends to favor the production of resistant sporangia; or, conversely, that a relatively long duration of high temperature tends to limit the production of resistant sporangia.

EFFECT OF AGGREGATE TEMPERATURE.[2] A computation of the aggregate temperatures, to which each series in experiment III was subjected, shows that there is an inverse relationship between tem-

[2] Degree hours: number of hours times temperature in degrees F.

perature and production of resistant sporangia. Arranged in tabular form these figures are presented in table 3. It may be concluded, then, that aggregate temperature is an important factor in determining the number of resistant sporangia produced in maltose-peptone agar cultures.

TABLE 3

	Series	Temperature in degree-hours per day (24 hours)	Average number of resistant sporangia
I	71° F.—24 hrs.	1704	72.27
II	71° F.—16 hrs. 79° F.— 8 hrs.	1768	58.90
III	71° F.— 8 hrs. 79° F.—16 hrs.	1832	43.75
IV	79° F.—24 hrs.	1896	38.25

It now seems possible to conclude that temperature is an important controlling factor in the production of resistant sporangia in cultures of *Allomyces arbusculus* grown on maltose-peptone agar, and that, within certain limits, the total amount of temperature to which cultures are subjected is of more significance than minimum temperatures, maximum temperatures, or degrees of fluctuation.

The results of the foregoing experiments suggest high aggregate temperature as a principal factor responsible for the frequent failures of resistant sporangia to form during the summer months.

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TWO NEW CHYTRID PARASITES OF CHYTRIOMYCES

JOHN S. KARLING

(WITH 1 FIGURE)

During the course of a study of an isolate of *Chytriomyces hyalinus* Karling (**5**) the sporangia of this chitinophyllic fungus became heavily infected with two other chytrids. The first of these parasites to be reported here relates to the eucarpic, rhizidiaceous genus *Rhizophydium*. It occurred first in great abundance on a pure culture of *C. hyalinus* which was growing on purified shrimp chitin, but later a few of its thalli were found on *C. aureus* also. In view of the latter discovery, inoculation tests were made at once to determine whether or not this fungus will infect other chitinophyllic chytrids. Heavily infected sporangia of *C. hyalinus* were repeatedly introduced into cultures of *Asterophlyctis sarcoptoides, Siphonaria variabilis, Obelidium mucronatum, Chytriomyces appendiculatus, Rhizophlyctis petersenii, Polychytrium aggregatum,* and *Rhizidium* sp., but none of these chytrids became infected. Likewise, cultures of *Achlya flagellata, Saprolegnia ferax, Aphanomyces* sp., and various species of *Pythium* were inoculated in the same manner, but no infection occurred. These results suggest that the parasite may be limited in host range to two species of *Chytriomyces*. It is significant to note that it has not infected *C. appendiculatus,* another chitinophyllic species of *Chytriomyces* from Virginia and Connecticut.

So far only six, or possibly seven, *Rhizophydium* parasites of other fungi have been reported: *R. carpophilum* on various aquatic Oomycetes (Zopf, **12**, and others), *R.* (*Mastigochytrium*) *Saccardiae* on the ascocarps of *Saccardia Durantae* (Lagerheim, **6**), *R. Pythii* on *Pythium complens* (de Wildeman, **10**), *R. fungicolum* on the hyphae of *Gloeosporium* (Zimmerman, **11**), *R. pollinis* on conidia of *Sclerospora graminicola* (Müller, **8**) and oospores of *Albugo bliti* (Melhus, **7**), *R. parasitans* on *Rhizophydium gonio-*

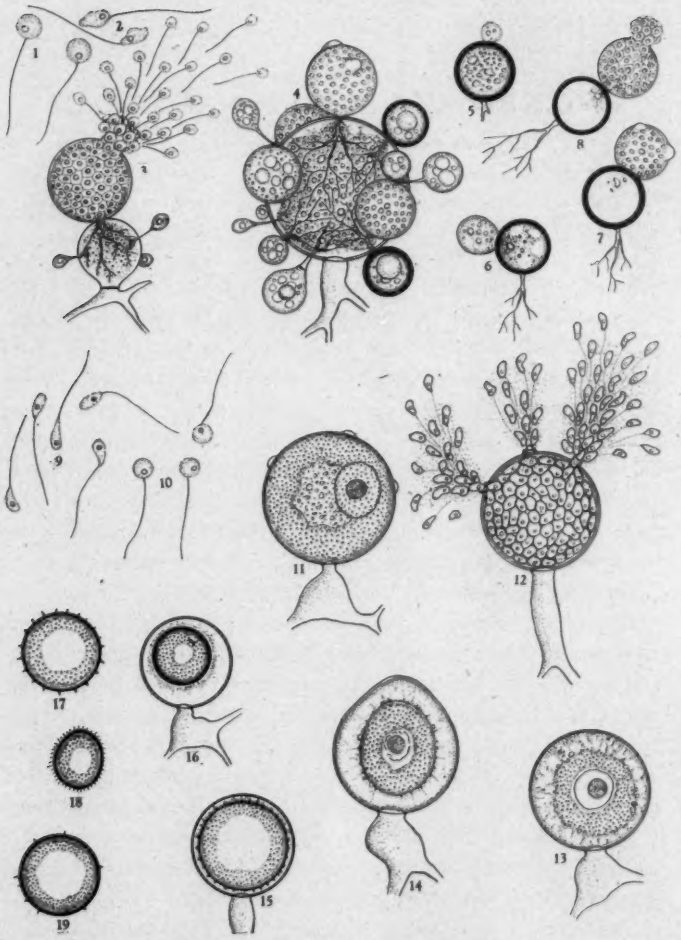

FIG. 1–8. *Rhizophydium Chytriomycii.* FIG. 9–19. *Rozella Chytriomycii.* 1, 2, free swimming and amoeboid zoospores; 3, emergence of zoospore, parasite's sporangium larger than that of host; 4, sporangium of *C. hyalinus* infected with eleven sporangia in various stages of development and two mature resting spores; 5–8, stages in germination of the resting spores; 9, free swimming resting spores; 10, zoospores immediately after coming to rest; 11, parasitized sporangium with three exit papillae, large central vacuole, and enlarged primary nucleus of host; 12, emergence of zoospores simultaneously from three exit papillae; 13, early stage of resting spore

sporum (Scherffel, 9), and *R. chytriophagum* on *Phlyctochytrium Aureliae* (Ajello, 1). Of the six, only *R. carpophilum* and *R. chytriophagum* are well known. Furthermore, it is not certain that the species reported by Müller and Melhus relates to *R. pollinis*. The sizes of the zoospores, sporangia, and resting spores are unknown for *R. Pythii;* resting spores are unknown for *R. Saccardiae* and *R. fungicolum,* and rhizoids are unknown in *R. parasitans*. Therefore, it is impossible to make extensive comparisons on morphological grounds between these species and the one on *Chytriomyces*. Except for *R. chytriophagum,* the zoospores of the *Chytriomyces* parasite are considerably smaller than those of the other species for which the spores have been observed. Although the parasites of *Chytriomyces* and *R. chytriophagum* have similar zoospores and resting spores, they differ markedly in the character of the rhizoids, and for this reason they are regarded as different species. Also, the fact that the parasite of *Chytriomyces* did not infect *Achlya, Saprolegnia, Aphanomyces,* and *Pythium* indicates that it has a different host range from those of *R. carpophilum* and *R. Pythii*. On these as well as on morphological grounds, we feel warranted in regarding it as a distinct species for which the name *R. Chytriomycii* is proposed.

The life cycle and developmental stages of *R. Chytriomycii* are shown in figures 1 to 8, and inasmuch as they do not differ markedly from those of other *Rhizophydium* species, it is unnecessary to describe them in detail. At least, no striking differences have been observed which warrant special attention. It may be noted, however, that the zoospores often germinate at considerable distances from the host cell with the result that the sporangia are often stalked (FIG. 3). Also, no evidence of sexuality has been seen in relation to resting spore development.

Rhizophydium Chytriomycii sp. nov.

Fungus parasiticus. Thallo numerosiis. Sporangii hyalinis, laevibus, sessilis aut caulis, sphericis, 8–30 μ, cum 1–3 papillis exeuntibus. Zoosporae

development, host nucleus in central vacuole; 14, later stage of development, host nucleus disintegrating; 15, mature echinulate resting spore which almost fills the host sporangium; 16, small smooth-walled resting spore; 17–19, resting spores freed by disintegration of host sporangium, showing variations in degree of echinulation.

hyalinis, sphaericis, 2–2.8 μ. Sporis perdurantibus laevibus, sphericis, 7–18 μ, pariete fuscis; germinantes ut prosporangia, parte interiori emergente ut zoosporangio membranum tenui ad superficiem sporae.

Thalli usually numerous, up to 20 on one host sporangium. Sporangia hyaline, smooth, sessile or stalked, spherical, 8–30 μ, with one to three exit papillae. Rhizoidal system finely branched, main axis up to 3 μ in diameter. Zoospores hyaline, spherical, 2–2.8 μ, with a minute, .5–.8 μ, hyaline refractive globule. Resting spores dark brown, spherical, 7–18 μ, with one or more large refractive globules; functioning as a prosporangium in germination.

Parasitic on *Chytriomyces hyalinus* and *C. aureus,* Candlewood Lake, Conn.

The second parasite found in *Chytriomyces* occurred intramatrically and relates to the genus *Rozella* of the family Olpidiaceae. It appears to be an obligate parasite which is limited in host range to *C. hyalinus.* All attempts to infect *C. aureus, C. appendiculatus, Obelidium mucronatum, Asterophlyctis sarcoptoides, Siphonaria variabilis, Rhizidiomyces hirsutus, Rhizophydium Chytriomycii, Rhizophlyctis petersenii, Nowakowskiella elegans,* and *Rhizidium* sp., with it have failed. Likewise, *Aphanomyces* sp., *Achlya flagellata, Saprolegnia ferax, Pythium anandrum, P. irregulare, P. splendens, P. ultimum, P. debaryanum, P. vexans, P. aphanidermatum, P. oligandrum, P. helicoides, P. graminicolum, P. aristosporum, P. acanthicum, P. tardicrescens,* and several unidentified species of *Pythium* have not become infected. Although these inoculation attempts do not include all of the hosts of the fourteen reported monosporangiate species of *Rozella* (see Karling, **2, 3, 4**), they nevertheless delimit this species from *R. Rhizophlyctii, R. Cladochytrii, R. irregularis,* and possibly *R. laevis, R. Endochytrii, R. cuculus,* and *R. Rhizophydii* as far as hosts are concerned.

Insofar as they are now known, most described monosporangiate species of *Rozella* are very similar in type of development, size, shape, and behavior of the zoospores, as well as in the color, size, shape, and echinulation of the resting spores. The parasite in *C. hyalinus* does not differ markedly in these characteristics from the other species, and it is therefore difficult to distinguish it sharply on morphological grounds. Unlike *R. Monoblepharidis,*

R. Rhipidii, R. Apodyae, R. irregularis, R. laevis, R. cuculus, R. Blastocladiae, R. Polyphagi, R. Cladochytrii, and *R. Endochytrii,* it does not cause hypertrophy of the host cell. In this respect it is similar to *R. Rhizophlyctii* and *R. Rhizophydii.* However, it does not infect the host of *R. Rhizophlyctii* and has slightly smaller zoospores than *R. Rhizophydii.* For these reasons we shall regard it as a distinct species under the name of *Rozella Chytriomycii* until further studies on this genus have proven it to be otherwise. At the same time, we realize fully that it, as well as many of the other *Rozella* parasites which have been described as valid species, may prove to be a physiological variety with a limited host range.

As is shown in figures 9 to 19, the development and life cycle of *R. Chytriomycii* are so similar to those of other monosporangiate species that very little can be added to the accounts already given. Although the parasite does not cause perceptible hypertrophy and distortion of the host cell, the primary host nucleus nevertheless becomes enlarged. In normal healthy thalli of the host, the primary nucleus does not divide until the sporangium is almost mature in size. Consequently, it becomes quite large and may be readily seen in the living condition as a hyaline, optically homogeneous, oval to spherical body with a large nucleole. In infected sporangia, the nucleus may appear even larger and often lies within or adjacent to a large central vacuole (FIG. 11). Such nuclei have never been observed to divide, and it may be assumed that the presence of the parasite prevents mitosis in addition to causing hypertrophy of the host nucleus. In several developmental stages of the resting spores (FIGS. 13, 14) the primary host nucleus was observed in the central vacuole, and by the time the spore wall was formed very little of the nucleus except the membrane and nucleole was visible (FIG. 14).

Rozella Chytriomycii sp. nov.

Fungus parasiticus; sporangia solitariis, longitudinem latitudinemque cellae matricalis in toto complentibus, sphericis, 10–40 μ, cum 1–3 papillis; pariete sporangiorum ex cella matricali fere non discernendo. Zoosporiis hyalinis, clavatis aut oblongatis, $3 \times 1.5\ \mu$, se in sporangio torquentibus antequam emergunt; natantibus emicatim, raro amoeboideis. Sporis perdurantibus, ovalibus, sphericis, 7–20 μ diametro, cum maxima gutta centrica et protoplasmate crasse granuloso; pariete fusco, spiculoso, raro laeve; germinatione non visa.

Sporangia solitary, hyaline, filling host cell and conforming with the latter in size and shape, usually spherical, 10–40 μ, with one to three exit papillae; wall of sporangium usually indistinguishable from that of host cell. Zoospores hyaline, oblong or slightly clavate, 3 × 1.5 μ, with a minute, .5–.7 μ, refractive globule; swirling in sporangium before emerging; darting about rapidly in swimming, rarely becoming amoeboid. Resting spores partly or almost completely filling host cell, oval or spherical, 7–20 μ, with large central vacuole, and coarsely granular cytoplasm; wall dark brown, rarely smooth, usually spiny or echinulate; germination unknown.

Parasitic in *C. hyalinus*, Candlewood Lake, Conn.

SUMMARY

Rhizophydium Chytriomycii and *Rozella Chytriomycii* are two new chytrid parasites which occur on and in the sporangia of *Chytriomyces*. The first parasite attacks *C. hyalinus* and *C. aureus*, while the second one is limited in host range to *C. hyalinus*. Extensive attempts to inoculate numerous other chytrids and larger fungi with these parasites have failed.

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A NEW SPECIES OF MYXOMYCETES

TRAVIS E. BROOKS

Three collections of an apparently undescribed species of Perichaena were made in Kansas on decaying leaves in August 1940. Later additional material was found while examining leaves collected in 1938 for *Physarum megalosporum* Macbr. A determined effort to find this species in the same area where the *P. megalosporum* had been found, and in several other Kansas localities, has yielded a number of excellent collections.

Perichaena syncarpon sp. nov.

Sporangiis sessilibus, sparsis vel stipatis, pulvinatis vel applanatis, 0.05–0.8 mm. latis, fulvo-brunneis, rufo-brunneis, vel nigris. Membrana exteriori cartilaginosa, firma, granulosa, in lobatis dehiscentis; interiori vix discreta, tenellula. Capillitio parce evoluto, cum vix ramosis tubis cavis. Sporis pallide luteis, subglobosis, spinulosis inaequalibus, 4–16 coalitis, 10–12 μ crassis. In foliis putrescentibus.

Plasmodia watery to opaque tan, ochraceous, or pinkish, sometimes somewhat opalescent, irregularly circular or oblong in outline, somewhat rugose, up to about 2 mm. across. Sclerotia minute, orange. Sporangia scattered, gregarious, crowded, or forming aethalia up to 2 mm. across, sessile on a broad base, pulvinate to applanate, often hemispherical or subglobose, sometimes forming short, straight or curved plasmodiocarps, circular in outline or polygonal from mutual pressure, yellowish-brown, reddish-brown, to black, often with a black margin, 0.05–0.8 mm. diam. Sporangium wall of two layers; the outer layer cartilaginous, firm, opaque, thickened with included deposits of dark granular matter, dehiscing into lobes along more or less prominent ridges, or dehiscing irregularly; the inner layer closely adhering to the outer, usually inconspicuous, membranous, pale yellow, transparent, sometimes somewhat iridescent, without deposits of refuse matter. Capillitium scanty, attached to the sporangium wall, consisting of sparingly branched yellow threads marked with minute, irregular, close-set constrictions and minute warts, 2–3.5 μ diam. Spores in mass golden yellow, pale yellow by transmitted light, adhering in clusters of 4–16, spinulose, more strongly spinulose on the outer surface as they lie in the cluster, 10–12 μ diam.

On decaying leaves of boxelder, catalpa, cottonwood, elm, giant ragweed, hackberry, and sunflower which have accumulated under

shrubbery or under dense associations of shrubbery, herbaceous plants, and grasses. Kansas; T. E. B. *495*, Geary Co., Aug. 21, 1938; T. E. B. *681*, Riley Co., Aug. 19, 1940; T. E. B. *734*, Saline Co., Aug. 22, 1940; T. E. B. *760*, Edwards Co., Aug. 4, 1940; T. E. B. *907*, Geary Co., Aug. 2, 1944; T. E. B. *920*, Geary Co., July 2, 1945 (Type collection); T. E. B. *928*, Geary Co., July 4, 1945; T. E. B. *930*, Douglas Co., July 7, 1945; T. E. B. *933*, Douglas Co., July 14, 1945; T. E. B. *934*, Douglas Co., July 19, 1945; T. E. B. *936*, Geary Co., July 21, 1945; T. E. B. *946*, Geary Co., Aug. 19, 1945; T. E. B. *948*, Geary Co., Aug. 19, 1945. All above collections were made by the writer. The frequency with which this species has been collected would indicate that it is not uncommon. Portions of the type collection have been distributed to the Mycological Collections of the United States Bureau of Plant Industry, Beltsville, Maryland, the Farlow Herbarium, Harvard University, Cambridge, Massachusetts, the Herbarium of Kansas State College, Manhattan, Kansas, the Herbarium of the New York Botanical Garden, New York, New York, the Herbarium of the State University of Iowa, Iowa City, Iowa, the Herbarium of the British Museum, London, England, and to Charles Meylan, St. Croix, Switzerland.

Perichaena syncarpon is readily distinguished from all previously described species of *Perichaena* particularly by the adherence of the spores in clusters. In the clustering of spores and the tendency to form aethalia this species shows a close affinity to *Minakatella longifila* G. Lister. It differs, however, in the definitely perichaenoid capillitium. Apparently *P. syncarpon* is intermediate between the species of *Perichaena* and *M. longifila*. This latter species is represented by a single collection of four small colonies or aethalia from Japan. Miss Lister (A Monograph of the Mycetozoa. Third edition, revised by Gulielma Lister. 1925) suggests that *Minakatella* has some affinity with *Perichaena*, but differs in the aethalioid habit and the smooth capillitium. The establishment of a species of *Perichaena* often having an aethalioid habit tends to support her idea.

The inconspicuousness of this species, its habitat on decaying leaves, and the possibility of a restricted distribution may account for the lack of previous collections. The material from Edwards, Riley, and Saline Counties was found beneath bushes on the under

surface of leaves in contact with the soil, and consists, for the most part, of colonies of crowded sporangia and aethalia. In other collections the habitat was apparently more favorable for the retention of moisture. The fruitings, consisting to a greater extent of distinct, scattered sporangia, were found abundantly on the surface of and within the leaf mat.

Sclerotia were found in abundance on leaves of 930 from Douglas Co. The day after the leaves were rewetted numerous small plasmodia were noted. These plasmodia were relatively inactive in that they moved about very slowly, and did not possess the back and forth flow of protoplasm characteristic of most plasmodia. Sclerotia have since been found in several of the collections. Plasmodia observed in the field have generally been pinkish or tan in color.

As is usual with most slime molds considerable variation occurs in this species. Although distinct sporangia, as well as those in colonies or aethalia, are generally pulvinate to applanate, small scattered sporangia are sometimes hemispherical or subglobose on a broad base, or rarely subglobose on a narrow base. Where sporangia are in contact the sporangium walls are often incompletely developed. Occasionally in aethalia the only vestige of these sporangium walls may be a single wall jutting toward the center of the aethalium from the margin, or a bar at the center of the aethalium extending from the base to the top. Sometimes sporangia or aethalia are depressed at the center. In several of the collections many of the smaller sporangia are shiny yellow with a very thin, transparent, yellowish sporangium wall through which the spore balls can readily be seen, due to the absence of refuse matter. Typically present are the raised lines along which the sporangium wall dehisces into lobes. Sometimes, especially in smaller sporangia, these dehiscence lines are weakly developed, or completely lacking. The capillitium has been scanty in all the material studied thus far. In smaller sporangia the capillitium is often completely lacking.

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LAWRENCE, KANSAS

NOTES AND BRIEF ARTICLES

WANTED

As a result of wartime losses, certain numbers of MYCOLOGIA have become exhausted. Among these are Volume 32, issue 1, and Volume 33, issue 2. Two dollars each will be paid for any copies of the above numbers.

FRED J. SEAVER,
Managing Editor

NOTES ON FLORIDA FUNGI

GOMPHIDIUS FOLIIPORUS Murrill. Singer's treatment of this species in Farlowia (**2**: 280) for July, 1945, is difficult to understand. Those of my readers who have received specimens from me can see at a glance that it is perfectly distinct from *Phylloporus rhodoxanthus* (Schw.) Bres. and, moreover, it is not a bolete at all.

In my species the cap is pale-reddish-fulvous; context pallid, becoming slightly bluish when cut; gills pallid, dark-blue when bruised, at length dark-brown; spores umbrinous in mass. See Mycologia **35**: 432. 1943, for full description. In my notes I stated that it was placed in *Gomphidius* only temporarily.

In *P. rhodoxanthus* the cap is reddish-yellow-brown to chestnut-brown; context yellowish, not bluing; gills deep-yellow, not bluing; spores yellowish in mass. Good descriptions are given by Atkinson, Kauffman and others.

How Singer can call my species simply a geographical race of *P. rhodoxanthus* is beyond me. Even when *G. foliiporus* turns a beautiful blue when wounded he waves it aside as of no special consequence. Not so, however, when he is describing *Gyrodon proximus* as distinct from *B. merulioides*.

BOLETUS FLAVIMARGINATUS Murrill. In Farlowia (**2**: 293) for July, 1945, Dr. Singer makes this species a synonym of *B. illudens* Pk. As I look at the two types on the table before me I cannot resist a smile at Dr. Singer's expense, and I believe he

would smile with me if he were here. The differences are so marked that even a small child could recognize them.

In my species the cap is reddish-fulvous, somewhat viscid; tubes bright-yellow, becoming greenish with age; spores about 14 ×4–5 μ; stipe smooth or delicately reticulate above. See Mycologia **31**: 110. 1939.

In Peck's species the cap is typically yellowish-brown, dry; tubes not so bright-yellow and soon becoming darker; spores about 11 × 4.5 μ; stipe coarsely reticulate, usually entirely to the base. See Rep. N. Y. State Mus. **50**: 108. 1897. Coker's pl. 35 shows the coarsely reticulated stipe very well. See Boletaceae of N. C. by Coker and Beers.

GYROPORUS ROSEIALBUS Murrill.– In Farlowia for July, 1945, Singer makes this species a synonym of my *G. subalbellus*, described in 1910 from Ocean Springs, Miss. With the types of the two species on the table before me, I maintain that they are distinct and have essentially the characters assigned to them in the original descriptions, although I now know much more about the beautiful *G. roseialbus* than I did at first. It is common about Gainesville, where I have found specimens 10 cm. broad with stems 8 cm. tall and 4 cm. thick. Average hymenophores are considerably smaller, but all are rosy-white when young. The spores in both species are as originally described, those of *G. subalbellus* being ovoid and about 7 × 4.5 μ, while those of *G. roseialbus* are oblong-ellipsoid and 11–13 × 4–5 μ.—W. A. MURRILL.

GAINESVILLE, FLA.

ANNUAL MEETINGS: They will be held in conjunction with the A.A.A.S. meetings at St. Louis, Missouri, March 27–30, 1946.